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14. ABSTRACT

The breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* (*BRCA1/2*) are key components of the Fanconi anemia (FA)/ homologous recombination (HR) pathway of DNA repair.. Based on previous work in the Swisher and Kaufmann laboratories, we proposed to test the **hypothesis** that *two different conditions must be met for ovarian cancer to be hypersensitive to platinum and PARP inhibitors: The FA/HR pathway must remain disabled and NHEJ must remain intact and functional.* Although we proposed two aims, the aim in previously banked specimens was removed before the present grant was awarded, leaving us with the following aim: **Correlate biomarkers of HR deficiency and NHEJ pathway integrity in pre-treatment biopsies with response to a PARPi in a prospective single-agent PARPi phase 2 clinical trial in sporadic ovarian carcinoma.** We obtained blood and tissue specimens from the phase 2 rucaparib trial (ARIEL2, ClinicalTrials.gov identifier NCT01891344) and completed sequencing of 75 DNA repair genes on blood and tumor samples from ARIEL2 We found that HR mutations and methylation both predicted response to rucaparib and these findings are being prospectively followed up in the phase 3 trial ARIEL3.

15. SUBJECT TERMS

ovarian cancer, drug resistance, rucaparib, phase 2, DNA repair, homologous recombination, nonhomologous end-joining (NHEJ), poly(ADP-ribose) polymerase, BRCA1, BRCA2, PARP1

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Table of Contents

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INTRODUCTION

Poly(ADP-ribose) polymerase (PARP) is an abundant nuclear enzyme that regulates five different DNA repair pathway. Building on preclinical observations that defects in homologous recombination (HR) repair, which are found in 30-50% of ovarian cancers, sensitize cells to killing by PARP inhibitor, five separate phase 3 trials involving PARP inhibitors have opened in ovarian cancer. In a recent decision the Food and Drug Administration approved the PARP inhibitor olaparib for women with recurrent ovarian cancer and inherited mutations in the *BRCA1* and *BRCA2* genes. Part 1 of the ARIEL2 biomarker clinical trial focused on enrolling predominantly women without inherited mutations in the *BRCA1* and *BRCA2* genes in order to develop a biomarker of PARPi responsiveness for non BRCA carriers. In collaboration with Scott Kaufmann (Mayo Clinic), this synergistic translational leverage project assessed multiple aspects of DNA repair pathway integrity in pretreatment biopsies from a large multi-institution phase 2 study of the PARP inhibitor rucaparib. In particular, the Swisher laboratory used massively parallel DNA sequencing to assess mutations in the HR pathway, the nonhomologous end-joining (NHEJ) pathway, PARP1 and other DNA repair genes that could impact response to PARP inhibitors. We also assessed promoter methylation of BRCA1 and RAD51C as another mechanism of HR deficiency.

Key words: ovarian cancer, drug resistance, rucaparib, phase 2, DNA repair, homologous recombination, nonhomologous end-joining (NHEJ), poly(ADP-ribose) polymerase, BRCA1, BRCA2, PARP1,

Overall Project Summary:

No changes were made to original approved statement of work.

The phase 2 clinical trial (ARIEL2 part 1) that provided samples for the correlative assays in the Kaufmann and Swisher laboratories (ClinicalTrials.gov identifier NCT01891344) completed enrollment in November of 2014. 204 patients were enrolled on ARIEL2 part 1 and all pre-treatment and archival biopsies were collected by Clovis Oncology. DNA from blood was collected by Clovis Oncology and transmitted to the Swisher laboratory.

De-identified specimens from blood and tumor tissue was obtained for all ARIEL2 part 1 participants. We completed sequencing of these specimens using the BROCA-HR next generation targeted sequencing test. We also assessed promoter methylation of *BRCA1* and *RAD51C* as another mechanism of HR deficiency. These data are published along with the major findings of the trial in Lancet Oncology (manuscript and supplement appended to this report, PMID).

Preliminary data on response rates were so promising in ARIEL2 that rucaparib was assigned breakthrough designation by the U.S. FDA and Clovis decided to amend ARIEL2 to enroll a second cohort of 160 patients to provide additional data for FDA approval (ARIEL2 part 2) and to test our predictive biomarker in a more heavily pre-treated patient population. On 12/19/2016 FDA approval for rucaparib was announced as well as for the companion diagnostic CDxBRCA from Foundation Medicine. The approval is for women with recurrent ovarian cancer and

germline or somatic *BRCA1* or *BRCA2* mutation as identified on the tumor sequencing CDxBRCA test. Data provided from this DoD program that supported this FDA submission included the germline testing, which we performed using BROCA. These data were used to confirm the accuracy of the companion diagnostic by an orthogonal method.

We plan to combine the Swisher sequencing findings together with the Kaufmann NHEJ protein assessment to determine if the combined biomarker is more predictive for PARPi response than the sequencing data alone. Those combined data will be finalized in the next year and we expect to publish at least one additional paper.

Key findings are detailed in the attached Lancet Oncology manuscript and supplement and briefly summarized here:

1. Ovarian carcinoma with somatic *BRCA* mutations have an equally high response rate to those associated with germline *BRCA* mutations and a similar duration of response.
2. All *BRCA* mutated cases had high LOH.
3. High genomic loss of heterozygosity was associated with improved progression-free survival, better overall response, and longer duration of response in *BRCA* wildtype cases compared to those with low LOH.
4. Mutations in some other HR genes, in particular *RAD51C* and *RAD51D*, also confer sensitivity to rucaparib.
5. Promoter hypermethylation of *RAD51C* and *BRCA1* in ovarian carcinomas is mutually exclusive with mutation of these genes and is associated with high response rates (75% and 52% resopectively).
6. One-third of cases with *BRCA1* methylation at diagnosis had lost methylation in the pre-treatment biopsy. Loss of *BRCA1* methylation may be a mechanisms of PARP inhibitor and platinum resistance. Therefore if methylation is to be used to select patients for PARP inhibitor therapy, it needs to be assessed in a recent biopsy.
7. The LOH algorithm was further refined based on the ARIEL2 response data and that algorithm is being assessed in the placebo controlled blinded phase 3 trial (ARIEL3) of rucaparib as maintenance therapy following platinum therapy for recurrent ovarian cancer.

Key research accomplishments

- We have identified predictors of PARPi response in women without germline BRCA mutations and platinum sensitive ovarian carcinoma including high LOH, methylation of BRCA1 and RAD51C, and mutations in key HR genes. This clinical predictor is being prospectively tested in a randomized phase 3 double blind placebo controlled trial of rucaparib as maintenance therapy in women with recurrent platinum sensitive ovarian cancer (ARIEL3, NCT01968213)

Conclusions

- Rucaparib has proven to have a high response rate in BRCA mutated ovarian cancer, including both somatic and germline mutations, which has led to recent U.S. FDA approval of this drug and the companion diagnostic (CDxBRCA) which was developed in the ARIEL2 clinical trial.
- Rucaparib also has clinical activity in a subset of high grade ovarian carcinoma without BRCA mutations.
- High genomic LOH is a marker of homologous recombination deficiency in ovarian cancer and may be useful to select patients who have ovarian carcinomas without BRCA mutations that are more likely to respond to a PARP inhibitor. The LOH algorithm developed in this study is being prospectively evaluated in the phase 3 trial ARIEL3.
- LOH is one marker of homologous recombination deficiency, but there are likely more precise ways to identify these cases. We are currently exploring other algorithms combining mutation, methylation and protein expression which we will compare with the LOH algorithm in the ARIEL2 population.

Publications

Scott CL, Swisher EM, Kaufmann SH.
Poly (adp-ribose) polymerase inhibitors: recent advances and future development. *J Clin Oncol* 2015 April 20;33(12):1397-406. PMID:25779564
Funding from this DoD award supported the collaboration between Dr. Swisher and Dr. Kaufmann in understanding predictors of PARP inhibitor responsiveness which is the major thrust of this OCRP proposal.

Swisher EM, Lin KK, Oza AM, Scott CL, Giordano H, Sun J, Konecny GE, Coleman RL, Tinker AV, O'Malley DM, Kristeleit RS, Ma L, Bell-McGuinn KM, Brenton JD, Cragun JM, Oaknin A, Ray-Coquard I, Harrell MI, Mann E, Kaufmann SH, Floquet A, Leary A, Harding TC, Goble S, Maloney L, Isaacson J, Allen AR, Rolfe L, Yelensky R, Raponi M, McNeish IA. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. Lancet Oncology. 2016 Nov 28. pii: S1470-2045(16)30559-9. doi: 10.1016/S1470-2045(16)30559-9. [Epub ahead of print] PMID:27908594

Abstracts and presentations

Identification of germline and somatic alterations in homologous recombination pathway genes in high grade ovarian carcinomas and response to the PARP inhibitor rucaparib in ARIEL2, Elizabeth Swisher, Clare Scott, Kevin K. Lin, Maria Harrell, James X. Sun, Sandra Goble, Amit Oza, Robert L. Coleman, Gottfried Konecny, Anna V. Tinker, David M. O'Malley, Rebecca

Kristeleit, Ling Ma, James Brenton, Katherine Bell-McGuinn, Ana Oaknin, Alexandra Leary, Elaina Mann, Heidi Giordano, Roman Yelensky, Mitch Raponi, Iain McNeish, accepted for oral presentation, AACR Ovarian Cancer Meeting, Orlando, FL, October, 2015

Results of ARIEL2: a Phase 2 trial to prospectively identify ovarian cancer patients likely to respond to rucaparib using tumor genetic analysis, **:** Iain McNeish, Amit Oza, Robert L. Coleman, Clare Scott, Gottfried Konecny, Anna Tinker, David O'Malley, James Brenton, Rebecca Kristeleit, Katherine Bell-McGuinn, Ana Oaknin, Kevin Lin, Mitch Raponi Heidi Giordano, Lara Maloney, Sandra Goble, Lindsey Rolfe Roman Yelensky, Andrew Allen, and Elizabeth Swisher, plenary presentation at ASCO, Chicago, IL, June 2015

Tumor BRCA mutation or high genomic LOH identify ovarian cancer patients likely to respond to rucaparib: interim results for ARIEL2 clinical trial, Elizabeth Swisher, MD, Amit Oza, MD, FRCPC, MBBs, Robert L. Coleman, MD, FACOG, FACS, Clare Scott, MB BS PhD, FRACP, Kevin Lin, PhD, Erin Dominy, BS, Lara Maloney, BA, Sandra Goble, MS, Roman Yelensky, PhD, and Iain McNeish, MD, PhD, MRCP, presented at Society of Gynecologic Oncology Annual Meeting as Late breaking Abstract, Chicago, IL, March, 2015

Inventions, patents and licenses

Nothing to report.

Reportable Outcomes

Rucaparib has proven to have a high response rate in BRCA mutated ovarian cancer, including both somatic and germline mutations, which has led to recent U.S. FDA approval of this drug for patients with relapsed BRCA-mutated ovarian cancer after second line therapy. The accompanying companion diagnostic (CDxBRCA) developed by Foundation Medicine is the first next generation sequencing test to be approved as a companion diagnostic.

Other achievements

Nothing to report.

Appendices

1. Clinical Cancer Research manuscript
2. Lance Oncology Manuscript and supplementary data.

Review

Clinical Cancer Research

Biomarkers of Response and Resistance to DNA Repair Targeted Therapies

Elizabeth H. Stover[1], Panagiotis A. Konstantinopoulos[1], Ursula A. Matulonis[1], and Elizabeth M. Swisher[2]

Abstract

Drugs targeting DNA damage repair (DDR) pathways are exciting new agents in cancer therapy. Many of these drugs exhibit synthetic lethality with defects in DNA repair in cancer cells. For example, ovarian cancers with impaired homologous recombination DNA repair show increased sensitivity to poly(ADP-ribose) polymerase (PARP) inhibitors. Understanding the activity of different DNA repair pathways in individual tumors, and the correlations between DNA repair function and drug response, will be critical to patient selection for DNA repair targeted agents. Genomic and functional assays of DNA repair pathway activity are being investigated as potential biomarkers of response to targeted therapies. Furthermore, alterations in DNA repair function generate resistance to DNA repair targeted agents, and DNA repair states may predict intrinsic or acquired drug resistance. In this review, we provide an overview of DNA repair targeted agents currently in clinical trials and the emerging biomarkers of response and resistance to these agents: genetic and genomic analysis of DDR pathways, genomic signatures of mutational processes, expression of DNA repair proteins, and functional assays for DNA repair capacity. We review biomarkers that may predict response to selected DNA repair targeted agents, including PARP inhibitors, inhibitors of the DNA damage sensors ATM and ATR, and inhibitors of nonhomologous end joining. Finally, we introduce emerging categories of drugs targeting DDR and new strategies for integrating DNA repair targeted therapies into clinical practice, including combination regimens. Generating and validating robust biomarkers will optimize the efficacy of DNA repair targeted therapies and maximize their impact on cancer treatment. *Clin Cancer Res; 22(23); 1–10.* *©2016 AACR.*

Introduction

Normal and cancer cells rely on multiple DNA damage response (DDR) pathways specialized to repair specific forms of DNA damage (Table 1; refs. 1–4). Key pathways include base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination repair (HRR), nonhomologous end-joining (NHEJ), and interstrand crosslink repair (ICL). If canonical repair pathways are deficient, or repair is unsuccessful, error-prone alternative pathways may be employed (e.g., alt-NHEJ, single-strand annealing, or translesion synthesis; refs. 1–4).

DNA repair targeted therapies exploit DNA repair defects in cancer cells to generate *synthetic lethality* (cell death resulting from simultaneous loss or inhibition of two critical functions; for example, cancer cells defective in one DNA repair pathway rely on alternate repair pathways; inhibition of a second repair pathway then results in cell death, an effect which selectively targets repair-deficient cancer cells; refs. 5, 6). DNA repair defects vary by cancer type. For example, approximately 50% of ovarian carcinomas (OC) exhibit dysfunctional HRR (7–10), colon and endometrial cancers are enriched in MMR defects (11), bladder cancers have frequent NER mutations (12), and testicular germ cell tumors may be functionally deficient in NER and other DDR pathways (13, 14). Many biomarkers for response to DNA repair targeted therapies reflect specific alterations in DDR pathways or genomic signatures resulting from aberrant repair.

Cytotoxic chemotherapies induce particular forms of DNA damage that trigger specific repair pathways. Therefore, cancers with DNA repair deficiencies show increased sensitivity to certain chemotherapeutics. For instance, OC patients with germline *BRCA1* or *BRCA2* (*BRCA1/2)* mutations (HRR deficiency) and bladder cancer patients with somatic *ERCC2* mutations (NER pathway) are more sensitive to platinum agents (15), likely due to decreased capacity to repair platinum-induced DNA damage. HRR and BER deficiencies sensitize cancer cells to topoisomerase-I inhibitors (e.g., topotecan), whereas HRR and NHEJ deficiencies sensitize to topoisomerase-II inhibitors (e.g., doxorubicin and etoposide; ref. 16).

HRR deficiency confers sensitivity to inhibitors of the PARP enzyme, which is vital to several DNA repair pathways, including BER and NHEJ. Developing biomarkers of DDR function and correlating DNA repair capacity with sensitivity to targeted agents is critical to optimizing efficacy of targeted DNA repair drugs. In this review, we describe candidate biomarkers of response (and resistance) to DNA repair targeted therapies. Genomic sequencing studies have demonstrated frequent DDR alterations in diverse cancers, suggesting that DNA repair targeted agents may be broadly active in cancer therapy and highlighting the need for accurate biomarkers of response (17, 18).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Table 1. DDR pathways and associated signaling pathways

Mechanism	Type of damage	Function	Key genes	Inhibitors of pathway proteins
DNA damage recognition, signaling, and checkpoints				
DNA double-strand break recognition	– Double-strand breaks (DSB)	– Recognition of DNA damage and recruitment of repair machinery	MRN complex: *MRE11, RAD50, NBN* (NBS1) *RBBP8* (CTIP) *PARP1*	PARP inhibitors MRE11 inhibitors (e.g., mirin)
DNA repair checkpoints	Various	– Coordination of cell cycle with DNA repair – Induction of cell death for irreparable lesions	*ATM* *ATR* *CHEK1* (Chk1) *CHEK2* (Chk2) *TP53* (p53) *H2AFX* (Histone H2A.X)	ATM inhibitors ATR inhibitors Chk1/2 inhibitors Wee1 inhibitors
Cell-cycle checkpoints	Various	– Regulate cell cycle to allow time for DNA repair activities and coordinate repair with progression through cell cycle	Genes encoding cyclin/CDK proteins	CDK inhibitors
Replication stress response	– Slowing/stalling of replicative DNA polymerase progression due to a variety of cellular stresses, resulting in stalled replication forks.	– Stabilization of stalled replication forks and DNA repair to enable replication restart. If this fails, DNA damage (e.g., DSBs) can result.	*RPA1, RPA2* *ATR* *ATRIP* *CHEK1* (Chk1) *TOPBP1*	ATR inhibitors Chk1 inhibitors
DNA repair pathways				
Direct repair	– Base modifications, including O6-methylguanine, 1-methyladenine, 3-methylcytosine, and N-methylated adenosine and cytosine – Examples: alkylating agents	– Direct repair of modified bases by enzymatic processes: demethylation	*MGMT* *ALKBH1*	MGMT inhibitors
Base excision repair (BER) – Short patch repair – Long patch repair – Single-strand break repair	– Damaged and modified bases – Single-strand breaks – Examples: radiotherapy, alkylating agents	– Excision of damaged base to generate a basic site, followed by nicking, resynthesis, and single-strand break repair (SSBR)	*OGG1* *NEIL1, NEIL2, NEIL3* *APEX1* *PARP1, PARP2* *XRCC1* *POLB* *LIG1* *LIG3* *FEN1* *PNKP* *MUTYH*	APE-1 inhibitors PARP inhibitors PNKP inhibitors POLB inhibitors
Nucleotide excision repair (NER) – Transcription-coupled NER – Global NER	– Bulky DNA adducts – Inter- and intrastrand crosslinks – Examples: platinum agents, ultraviolet (UV) light	– Damage recognition and unwinding of local DNA, nuclease excision, resynthesis, and SSBR	*RAD23B* *DDB1* *RPA1, RPA2* *ERCC1, ERCC2* (XPD), *ERCC3, ERCC5, ERCC6, ERCC8* *GTF2H1, GTF2H2, GTF2H4, GTF2H5, GTF2F2* *CDK7* *MMS19* *MNAT1* *XPA, XPC* *CCNH* *PCNA* *RFC1*	
Mismatch repair (MMR)	– Base mismatches (single nucleotide mutations and small insertions/deletions) – Examples: alkylating agents, replication errors	– Recognition and removal of mismatched base followed by resynthesis of correct base and SSBR	*MLH1* *MLH3* *MSH2* *MSH6* *PMS2*	
Homologous recombination repair (HRR)	– Double-strand breaks – Examples: radiation, topoisomerase inhibitors, cisplatin	– Unwinding and resection at DSB to generate single-strand end, strand invasion, homologous recombination with sister chromatid, resynthesis, and resolution. Results in exact repair using sequences from sister chromatid.	*BRCA1* *BRCA2* *RAD51, RAD52* *TP53BP1* *RBBP8* (CTIP) *EXO1* *RPA1, RPA2* *BLM* *PALB2* MRN complex: *MRE11, RAD50, NBN* (NBS1)	RAD51/2 inhibitors BLM inhibitors

(Continued on the following page)

Table 1. DDR pathways and associated signaling pathways (Cont'd)

Mechanism	Type of damage	Function	Key genes	Inhibitors of pathway proteins
Interstrand crosslink repair (ICL)	– Interstrand crosslinks – Examples: platinum agents, nitrogen mustards, mitomycin C	– Crosslinks are excised and then repaired by HRR (or other mechanisms)	*BRCA2* *FANCA* *FANCB* *FANCC* *FANCD2* *FANCE* *FANCG* *FANCF* *FANCI* *FANCL* *BRIP1* *FANCM* *FAAP20* *FAAP100*	
Nonhomologous end joining (NHEJ) – Classical NHEJ – Alternative NHEJ or microhomology-mediated end joining	– Double-strand breaks – Examples: radiation, topoisomerase inhibitors, cisplatin	– Processing and re-ligation of double-strand break ends. Error prone due to processing steps and because the homologous template is not used for repair.	*PRKDC* (DNA-PKcs) *XRCC5* (Ku80), *XRCC6* (Ku70) *LIG4* *XRCC4* *POLQ* *NHEJ1* *DCLRE1C* (Artemis) *PARP1, PARP2* *XRCC1*	DNA-PK inhibitors
Single-strand annealing	– Double-strand breaks	– Homology-mediated repair of repetitive regions	*RPA1, RPA2* *RAD52*	
Translesion synthesis (damage bypass rather than repair)	– DNA adducts – Examples: platinum agents, UV light	– Error-prone polymerases synthesize DNA past regions of damage, especially bulky DNA adducts	*POLH*	

NOTE: DDR pathways and therapeutic targets. Summary of DNA damage repair pathways, their functions, and key proteins, as well as classes of DNA repair targeted agents that inhibit different pathways.
Abbreviation: DNA-PKcs, DNA-dependent protein kinase catalyic subunit.

HRR Deficiency Assays as Biomarkers of PARP Inhibitor Response

PARP inhibitors (PARPi) are selectively lethal to HRR-deficient cells (19, 20). Synthetic lethality may be mediated by PARPi impairment of BER, although at least six potential mechanisms of action have been suggested, including alterations in NHEJ, alternative end joining, and DNA repair protein recruitment; PARP trapping at the replication fork is particularly significant, generating increased double-strand breaks (DSB) and dependence upon HRR (10, 21). Different PARPi may vary in their specificity for PARP enzymes and PARP trapping activity. Identifying the clinically relevant mechanisms of PARPi activity and resistance will be important to selecting optimal biomarkers.

Olaparib was the first FDA-approved PARPi after clinical trials showed benefit in OC and other cancers, primarily in patients with germline *BRCA1/2* mutations (21–24). Olaparib is approved in the United States for patients with recurrent OC who have a germline *BRCA1/2* mutation and in whom at least 3 lines of therapy have failed. Clinical trials of various PARPi in diverse clinical contexts and in combination with several agents are summarized in Supplementary Table S1. When PARPi are used as a single agent, resistance typically develops in months, though occasional sustained responses are observed (24).

The success of PARPi in patients with germline *BRCA1/2* mutations and clinical trials showing better response rates in cancers with germline and somatic *BRCA1/2* mutations than in those without have confirmed that damaging *BRCA1/2* mutations (suggesting HRR deficiency) are an important biomarker for PARPi sensitivity (24). Restoration of HRR function by somatic reversion of germline *BRCA1/2* mutations confers platinum and PARPi resistance in OC (25, 26).

Because sensitivity to platinum and PARPi are both associated with HRR defects, platinum sensitivity has been used as a surrogate for HRR deficiency in OC. However, some patients with platinum sensitivity do not respond to PARPi, and trials of PARPi in unselected patients have produced responses in a subset of patients with platinum resistance (27). Hence, platinum and PARPi responsiveness are not always concordant. Variability in DNA repair function may underlie this complexity: NER gene mutations are associated with platinum sensitivity in OC patients and cell lines but exhibit resistance to PARPi *in vitro* (28), and PARPi resistance mechanisms, such as loss of TP53BP1 or REV7, may be associated with platinum sensitivity (29, 30).

Clinically feasible, accurate biomarkers for response and resistance to PARPi are needed. Numerous assays for HRR deficiency are available, each with advantages and disadvantages (Table 2) as well as varying capability to predict PARPi response that must be tested in prospective clinical trials.

Targeted sequencing

Targeted multiplex sequencing can identify germline and somatic mutations in DNA repair genes that result in increased

Table 2. Assays for HRR function

Assay	Examples	Advantages	Disadvantages
Targeted sequencing	– Specific gene panels – Whole-exome sequencing	– Can assess many DNA repair genes simultaneously – Can identify both somatic and germline alterations	– Must know *a priori* which genes will have clinical impact – Functional impact of many variants uncertain
Whole-genome sequencing	– Mutational signatures of DNA repair deficiency	– Not reliant on identifying mutations in specific genes	– Expensive – Requires advanced bioinformatics
Copy number analyses	– Loss of heterozygosity – Telomeric allelic imbalance – Large-scale transitions	– Not reliant on identifying mutations in specific genes – Some commercial assays are in clinical development	– Historical, rather than dynamic, biomarker
Gene-expression profiling	– Expression arrays – RNA-Seq – NanoString – Quantitative RT-PCR	– Global readout from many upstream inputs and genetic alterations – Tractable for use in the clinic – Potential for real-time readout	– Poorly reproducible between studies – Can be confounded by tumor/normal mixtures – Requires tissue biopsy for dynamic readout
Protein expression assays	– Immunohistochemistry (IHC) – Mass spectrometry–based methods – Protein chips	– IHC is applicable to small clinical samples – Can reflect functional impact of alterations in DNA and RNA – Potentially dynamic readout	– Difficult to identify reliable markers of DNA repair activity – Depending on the assay, can be poorly reproducible – Require tissue biopsy for real-time assessment
Functional assays	– RAD51 foci formation – γ-H2AX – PARylation – Phospho-NBS1 (NBN) – DNA fiber assay – RPA foci	– Directly reflect DNA repair capacity – Integrate functional effects of multiple levels of cellular alterations (DNA, RNA, protein)	– Difficult to apply in clinical practice—most require fresh tissue and exposure to DNA damage

NOTE: Assays for HRR. Approaches for identifying deficient HRR in cancer, which may affect response to DNA repair targeted therapies.
Abbreviation: RT-PCR, reverse transcriptase PCR.

or decreased HRR. Although *BRCA1/2* mutations are the most prevalent biomarkers in PARPi trials, PARPi responses observed in some *BRCA1/2* wild-type patients (27) suggest that alterations in other HRR genes may also confer sensitivity. BROCA is a targeted next-generation sequencing assay that was used to identify damaging mutations in at least one of 13 HRR genes (*BRCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*) in one third of advanced OC; HRR mutations strongly correlated with platinum sensitivity and overall survival (8). Whole-exome sequencing (WES) provides targeted sequencing of most exons, including DNA repair genes, but is not commonly applied in clinical practice. A PARPi clinical trial in metastatic prostate cancer used WES, with an 88% response rate in the one third of patients with HRR gene mutations (31). Variants of uncertain significance (VUS), sequence alterations whose functional significance is unknown, present a particular challenge in clinical practice. Many resources are available to help infer the consequences of VUS [e.g., variant classification databases such as ClinVar, population databases that provide variant frequencies such as the Exome Aggregation Consortium (ExAC) and the Exome Sequencing Project (ESP), and online tools for prediction of variant pathogenicity (PolyPhen-2, SIFT, MutationTaster)]. Nevertheless, the functional and clinical relevance of many VUS remains uncertain, and predictive functional assays for DNA repair genes are needed to improve variant interpretation.

"Genomic scars" (mutational signatures and alterations in genome structure)

Genomic scars represent accumulated patterns of DNA damage and repair identified by genomic profiling (32–34). For example, because cells deficient in HRR rely on more error-prone DNA repair pathways such as NHEJ, large genomic deletions and loss of heterozygosity (LOH) are typical of an HRR phenotype.

Mutational signatures describe genome-wide patterns of nucleotide alterations reflecting historical exposures to DNA damage and repair. Specific mutational signatures are associated with defects in various DDR pathways, including *BRCA1/2* (tandem duplications, microhomology mediated deletions) NER (UV light signature), mismatch repair (MMR; microsatellite instability), and *POLE* (ultramutation signature; ref. 35). Microsatellite instability is a useful clinical test to identify MMR deficiency, which can suggest an underlying inherited disorder (Lynch syndrome). Other mutational signatures may have increasing diagnostic or clinical utility as whole-exome and genome sequencing become more prevalent, such as using mutational signatures suggestive of *BRCA1/2* mutations to identify PARPi sensitivity.

Large-scale disarray in chromosome structure is common in HRR-deficient cancers and may be quantitated by several assays including: (i) LOH, patterns of loss of one allele at many sites across the genome, via deletion or copy number neutral LOH; (ii) telomeric allelic imbalance (TAI), allelic imbalance near telomeres; and (iii) large-scale state transitions (LST), chromosomal breaks between adjacent regions of $\geq$ 10 Mb. LOH quantification correlates with platinum response in OC (36). TAI scores correlate with platinum response in breast cancer and OC and are associated with *BRCA1/2* mutations (37). LST is associated with *BRCA1/2* alterations in basal-like breast cancer (38). The three scores (LOH, TAI, LST) show a strong correlation with one another (39).

Several HRR deficiency biomarkers using patterns of LOH are being tested as potential companion diagnostics in PARPi clinical trials. An LOH assessment using a sequencing assay from Foundation Medicine was tested as a prospective biomarker in a phase II PARPi clinical trial. In a trial of rucaparib in recurrent OC, response rates were 80%, 29%, and 10%, respectively, in patients with (i) germline and somatic *BRCA1/2* mutations, (ii) high LOH but no *BRCA1/2* mutation, and (iii)

low LOH and no BRCA mutation (40). This suggests that LOH can be used as a surrogate for HRR and predict PARPi response, a hypothesis being tested in multiple prospective trials (e.g., NCT02655016; Supplementary Table S1). Myriad Genetics also developed an HRR deficiency assay based on a combination of LOH, TAI, and LST, which predicts response to neoadjuvant platinum in triple-negative breast cancer (41), and is testing this assay prospectively in multiple PARPi trials. A randomized phase III trial of the PARPi niraparib as maintenance therapy following complete or partial response to platinum in women with recurrent ovarian cancers demonstrated benefit across all subgroups, including those with cancers that tested negative for HRD by the Myriad Genetics HRD assay, though the relative improvement in progression-free survival was greater in patients with HRD (42). Presumably, the genomic scars identified by these assays remain detectable even if functional HRR is re-established, for example, by reversion mutations or epigenetic changes. If true, then LOH profiling might be more predictive of PARPi responsiveness early in the disease course, before various resistance mechanisms have accumulated.

Gene and protein expression

Gene-expression signatures of DDR genes have been described that correlate with outcome and platinum response in ovarian, breast, and lung cancers (43–45). However, a meta-analysis of expression signatures in OC showed that this approach suffers from poor reproducibility (46). Alternatively, HRR protein levels or methylation, for example, by immunohistochemistry, may be useful in revealing dynamic HRR alterations. *BRCA1* promoter hypermethylation, which downregulates *BRCA1*, might contribute to HRR deficiency, and response rates of 52% were recently reported for *BRCA1* methylated ovarian cancers treated with the PARPi rucaparib (40). Reversal of *BRCA1* hypermethylation was observed in acquired platinum resistance in OC, suggesting that neoplastic cells may re-express silenced *BRCA1* as a resistance mechanism (9). *BRCA1* expression is further modulated by microRNAs, altering PARPi sensitivity (47). Many studies of HRR protein expression have been limited by small numbers or technical issues, with poor reproducibility or results inconsistent with current models of HRR signaling. Therefore, both gene and protein expression as a biomarker for PARPi or other DNA therapies requires further research and clinical validation (48).

Functional assays

Functional assays can quantitate DNA repair capacity and may provide the most dynamic, real-time readout of DNA repair but are clinically hampered by technical challenges such as the need for fresh tissue and a DNA-damaging stimulus (49). The *RAD51 focus formation assay* reflects activation of HRR machinery and has been applied to clinical samples. Fewer irradiation-induced RAD51 foci in *ex vivo* breast cancers and in OC ascites correlated with HRR defects (50, 51) and better response to neoadjuvant chemotherapy (52). *Gamma-H2AX foci* are increased in the presence of DNA DSBs; *foci of 53BP1* also mark DSBs and have been correlated with impaired DSB repair (49, 53). *Phospho-NBS1 (p-NBS1) (NBN)* marks activation of the MRE11–RAD50–NBN (MRN) complex, which mediates early processing of DSBs (54); nuclear p-NBS1 staining was shown to be feasible in biopsies in a clinical trial of the PARPi veliparib (55). *Intratumoral levels and activity of the PARP enzyme* are a good pharmacodynamic marker of PARPi activity but do not clearly correlate with clinical responses in patients (55). Similarly, PARP enzyme activity in peripheral blood lymphocytes is a good pharmacodynamic but not predictive biomarker in PARPi clinical trials (56, 57). *In vitro* cell line studies have shown that HRR deficiency results in hyperactivation of PARP and increased levels of PAR polymers, suggesting that hyperactivated PARP or lower PAR levels may be a marker of PARPi sensitivity or resistance, respectively (58, 59). Clinically, wide variations in PARP activity have been observed among patients, which may limit its utility as a predictive biomarker (60). Changes in PARylation levels have also been used as pharmacokinetic markers of effective PARPi activity (49). *Assays for replication stress* include the DNA fiber assay (61) and replication protein A (RPA) foci. Certain forms of DNA damage (e.g., bulky adducts) result in stalling of replication forks. Emerging evidence suggests that replication fork stabilization might be a mechanism of resistance to platinum and PARPi independent of HR dysfunction; for instance, Fanconi Anemia group D2 protein (*FANCD2*) and Pax-interacting protein 1 (*PAXIP1*, *PTIP*) can stabilize stalled replication forks, enabling bypass of the blockage and cell survival (62).

These assays represent a wide range of approaches for predicting PARPi response. Selection of optimal, clinically feasible assays awaits validation from clinical trials (Supplementary Table S1).

ATM/ATR Inhibitors and Biomarkers

ATM and ATR kinases are critical components of the early response to DNA damage and activation of cell-cycle checkpoints (reviewed in refs. 63–66). ATM and ATR collaborate with the checkpoint proteins Chk2 and Chk1, respectively, to arrest the cell cycle and allow time for DNA repair. Studies suggest that inhibiting ATM and/or ATR might increase sensitivity to DNA damage. This sensitization may be particularly profound in cells with deficient DNA repair or increased replication stress.

ATM, ATR, and dual ATM/ATR inhibitors have been developed, with several in clinical trials (Table 3, Supplementary Table S1). VX-970 (formerly VE-822) is a potent, selective ATR inhibitor that sensitizes cancer cells with defective DNA repair to chemotherapy (67). Clinical trials are underway combining VX-970 with chemotherapy in advanced malignancies. AZD6738 is another selective ATR inhibitor that is being tested in combination with several agents in phase I trials. AZD0156 is a selective ATM inhibitor that is being studied in combination with olaparib in a phase I trial in advanced cancer; several other ATM inhibitors are undergoing preclinical investigation. Combined ATM/ATR inhibitors are generally less specific, and are not active in clinical trials (reviewed in ref. 66).

Several biomarkers for response to ATM or ATR inhibitors have been proposed, though clinical data are limited. Alterations in the target kinases (ATM/ATR) or their protein complexes may confer sensitivity to ATM and/or ATR inhibitors (66, 68). DDR deficiencies may sensitize to ATM/ATR inhibitors due to increased reliance on DDR checkpoints (68). Alterations causing increased replication stress may enhance sensitivity to ATM/ATR inhibition, for example, *TP53* mutations, *CCNE1* (Cyclin E1) amplifications (69, 70), and mutations in oncogenic drivers such as *RAS* and *MYC* (71, 72). Alternative lengthening of telomeres (ALT), the maintenance of telomere length through an HRR-based mechanism as an alternative to telomerase, may be a biomarker of hypersensitivity to ATR inhibitors (73). Loss of ATRX, a chromatin remodeling protein, was associated with increased ALT in most cell lines (73), and may also predict response to ATR inhibition.

Table 3. DNA repair related therapies in preclinical and clinical development

Class	Target protein role in DDR	Classes of agents and examples of drugs in clinical trials	Clinical development phase and context	Potential biomarkers
PARP inhibitors	PARP detects single-strand DNA breaks and synthesizes a poly (ADP-ribose) chain (PAR) to recruit repair proteins	PARP inhibitors: – Olaparib – Rucaparib – Niraparib – Veliparib – Talazoparib	Olaparib: FDA-approved in recurrent ovarian cancer with germline *BRCA1/2* mutations Others in phase I–III trials in advanced solid malignancies, alone or in combination with chemotherapy, antiangiogenic agents, other targeted agents, and immunotherapy (Supplementary Table S1)	Assays for HRR deficiency (Table 2): – Exome sequencing of HRR genes – Mutational signatures – Copy number analyses – Gene and protein expression – Functional assays
ATM/ATR inhibitors	ATM/ATR: kinases that collaborate with Chk1/2 to arrest cell cycle, allowing time for DNA repair	ATR inhibitors: – VX-970 – AZD6738	VX-970, AZD6738: phase I/II trials combined with chemotherapy or radiation in advanced solid tumors	ATR alterations Alternative lengthening of telomeres ATRX loss DDR deficiency Replication stress
		ATM inhibitor: – AZD0156	Phase I trial ± PARPi in advanced malignancies	ATM alterations DDR deficiency Replication stress
		ATM/ATR inhibitors	Preclinical	
NHEJ inhibitors	DNA-PK: enzyme complex initiating repair of DSBs by NHEJ (DNA-PKcs = catalytic subunit)	DNA-PKcs inhibitors: – CC-122 – ZSTK474 – CC-115 – MSC2490484A – NU7026 – NU7441	MSC2490484A combined with radiation in an ongoing phase I trial CC-115 and CC-122 in phase I trials in advanced malignancies Preclinical	Under development
	DNA ligase IV: major DNA ligase enzyme in NHEJ	DNA ligase IV inhibitors		Under development
BER inhibitors	AP endonuclease 1 (APE-1) helps remove damaged bases in BER	APE-1 inhibitors: – Methoxyamine (TRC102) – Inhibitors of AP endonuclease activity (e.g., lucanthone)	Phase I and II combinations with pemetrexed/cisplatin or temozolomide in advanced solid tumors	HRR defects may increase sensitivity
	Bifunctional polynucleotide phosphatase/kinase (PNKP) adds or removes phosphates to DNA ends; also involved in NHEJ	PNKP inhibitors	Preclinical	Under development
	DNA polymerase beta (POLβ) synthesizes DNA in BER	POLβ inhibitors	Preclinical	Under development
MRN complex inhibitors (HRR)	MRN complex (MRE11A–RAD50–NBN) recognizes DSBs and initiates repair via protein recruitment; MRE11A mediates end resection	MRE11 inhibitors (e.g., mirin)	Preclinical	Under development
HRR inhibitors	RAD51: recombinase promoting homologous recombination	RAD51 inhibitors	Preclinical	Under development
	DNA helicases unwind DNA in several repair processes	Inhibitors of helicases (e.g., BLM, WRN, RECQL1)	Preclinical	Under development
Chk1/2 inhibitors	Chk1 and Chk2: cell-cycle checkpoint kinases. Chk1 promotes cell-cycle arrest at the G_2–M checkpoint and DNA repair. Chk2 promotes the G_1 checkpoint and DNA repair.	Chk1 inhibitors: – MK-8776 – GDC-0575 – PF00477736 – SCH 900776	GDC-0575: phase I with gemcitabine in advanced solid tumors and lymphoma	– Chk1 overexpression suggests sensitivity – RAD50 mutation reported in outlier response to Chk1 inhibitor + chemotherapy
		Chk1/Chk2 inhibitors: – AZD7762 – LY2606368	LY2606368: phase II in breast, ovarian, and metastatic prostate cancer and small cell lung cancer	
		Chk2 inhibitors	Preclinical	

(Continued on the following page)

Table 3. DNA repair related therapies in preclinical and clinical development (Cont'd)

Class	Target protein role in DDR	Classes of agents and examples of drugs in clinical trials	Clinical development phase and context	Potential biomarkers
p53 targeting	Wee1 kinase is involved in G_2-checkpoint signaling	Wee1 inhibitor: – AZD1775 (MK-1775)	AZD1775: phase I and II in advanced solid tumors and myeloid malignancies in combination with chemotherapy or PARPi	*TP53*-mutant tumors are more sensitive because they lack the G_1 checkpoint and rely on the G_2 checkpoint for DNA repair
CDK inhibitors		CDK4/6 inhibitors: – Palbociclib (PD-0332991) – Ribociclib (LEE011) – Abemaciclib (LY2835219) – G1T28	Palbociclib is FDA-approved. Palbociclib, ribociclib, and abemaciclib in phase I-III trials as single agent or in combination with chemotherapy or targeted agents in various solid tumors. – G1T28 is in phase I/II trials + chemotherapy in SCLC	– Loss of Rb and CCNE1 copy gains are associated with resistance to CDK4/6 inhibitors
		Multiple CDK inhibitors: – Dinaciclib (SCH-727965) (CDK1/2/5/9/12) – AT7519 (CDK 1/2/4/5/6/9) – SNS-032 (CDK2/7/9)	Dinaciclib: phase I/II alone or in combinations in solid tumors and hematologic cancers AT7519: phase I/II in solid tumors and hematologic cancers	– CCNE1-amplified tumors may be sensitive to CDK2 inhibitors
		CDK12 inhibitors	Preclinical	Under development

NOTE: For purposes of this table, specific agents were listed only if they are in current or recent clinical trials, but numerous additional molecules have entered clinical or preclinical development over the past decade. Active clinical trials were identified from a keyword search of clinicaltrials.gov (April–May 2016) limited to United States, phase I/II/III, age >18, and open studies.

NHEJ Inhibitors and Biomarkers

NHEJ is a second major pathway of DSB repair, along with HRR. Repair pathway choice between NHEJ and HRR is mediated by cell-cycle phase (HRR occurs during S phase, whereas NHEJ can proceed during all phases) and by active mediators of pathway choice such as TP53BP1, which promotes HRR and inhibits NHEJ (4). NHEJ is more error prone due to its end processing and religation mechanism resulting in nucleotide loss versus the conservative recombination of HRR using a normal DNA template to exactly replace the damaged region (1).

Inhibitors of several NHEJ proteins have been developed, and DNA-PKcs inhibitors are the most clinically advanced (Table 3; reviewed in refs. 74, 75). DNA-PKcs is the catalytic subunit of DNA-PK, a PI3K-related kinase similar to ATM and ATR. Phosphorylation of substrates by DNA-PK induces recruitment of repair proteins to DSBs and activation of checkpoints (75). CC-115 is a DNA-PKcs/mTOR inhibitor that has entered clinical trials (76), and CC-122 is a DNA-PK inhibitor (termed a "pleiotropic pathway modifier") in a phase I clinical trial (75). ZSTK474 is an ATP-competitive inhibitor of PI3K that also inhibits DNA-PK and has been tested in early phase clinical trials (75). NU7026 and NU7441 are selective ATP-competitive inhibitors of DNA-PK undergoing preclinical development (77, 78). DNA-PK inhibitors sensitize cancer cells to DSB-inducing chemotherapies or radiation in preclinical studies, so combination strategies may be considered; MSC2490484A is a DNA-PK inhibitor being combined with radiotherapy in a phase I trial (Supplementary Table S1).

Biomarkers of sensitivity to NHEJ inhibition have not yet been validated, although *in vitro* HRR-deficient cells exhibit enhanced sensitivity to DNA-PK inhibition, perhaps because they are more dependent on NHEJ for repair of DSBs (1). Synthetic lethality between DNA-PK loss and various DDR proteins has been observed in preclinical studies (1, 79).

Other DNA Repair Related Therapies

Several classes of DNA repair targeted agents have emerged in preclinical and early clinical studies (Table 3) that are outside the scope of this review, including inhibitors of AP endonuclease 1 (APE-1), bifunctional polynucleotide phosphatase/kinase (PNKP), DNA polymerase beta, RAD51, RAD52, and DNA repair associated helicases such as BLM. The checkpoint kinases Chk1 and Chk2 are intimately linked to the DNA-damage cell-cycle checkpoint mediated by ATM and ATR, and Chk1/Chk2 inhibitors are advancing in clinical trials (reviewed in refs. 2, 3, 80).

Several other classes of agents are closely linked to DDR, and many show enhanced activity in cells with DDR deficiencies. CDK inhibitors block cyclin-dependent kinases critical to cell-cycle progression, thereby impacting DNA repair that occurs during, and depends upon, specific phases of the cell cycle. These include inhibitors of CDK4/6 (e.g., palbociclib, which was recently FDA approved in metastatic breast cancer), CDK1/2/5/9, and CDK12. CDK12 promotes transcription of large RNAs including many HRR genes; inhibition of CDK12 has also been shown to downregulate HRR through transcriptional regulation (81, 82).

The p53 protein is critical to the DNA damage response via numerous functions, including activation of DDR, G_1–S arrest to allow DNA repair, and apoptotic cell death following irreparable DNA damage. Wee1 inhibitors enhance sensitivity to DNA-damaging agents, preferentially in p53-deficient cells that are more reliant on the G_2 checkpoint (83).

Biomarkers for most of these agents have yet to be determined (Table 3).

Future Perspectives

DNA repair targeted agents are rapidly affecting cancer therapy. PARP inhibitors have become the paradigm of synthetic lethality

and are expanding therapeutic options in many cancer types, but the >1,000-fold increase in sensitivity seen in cells deficient for BRCA1 or BRCA2 has not been matched by other DNA repair therapies. Nevertheless, genomic sequencing has revealed a previously underappreciated frequency of DNA repair aberrations across tumor types, suggesting that many patients with advanced malignancies may be candidates for DNA repair targeted therapeutics.

Several issues need to be addressed to optimize the clinical application of DNA repair targeted agents. Robust and clinically feasible biomarkers of response and resistance must be developed, necessitating comprehensive incorporation of potential biomarkers in clinical trials, technical standardization of biomarker assays, and systematic clinical data collection to correlate biomarker data with clinical responses. Promising predictive biomarkers must then be tested prospectively; for instance, *BRCA1/2* germline mutations were tested prospectively as biomarkers for PARPi response, and LOH-based HRR deficiency assays are embedded in ongoing PARPi trials. Additional research is required to identify and validate predictive biomarkers, particularly for DNA repair targeted therapies beyond PARPi, and, at some point, head-to-head comparisons will be required to compare biomarkers in situations where multiple tests are available. Finally, minimally invasive "blood biopsies" of circulating tumor cells or plasma cell-free DNA may contain sufficient genomic information to infer DDR phenotypes of solid tumors in the absence of a tissue biopsy (84); incorporating blood biopsies alongside tumor-based assays will allow rapid assessment of this promising biomarker strategy.

Optimal application of DNA repair targeted agents may require combination strategies (Supplementary Table S1). Maximizing the cellular dependency on DDR inhibition often requires a DNA damage insult, such as chemotherapy or radiation (3). Additionally, responses to DNA repair targeted agents may be enhanced by targeting alternative DDR pathways upon which cells rely when the canonical repair pathway is impaired, potentially resulting in increased efficacy from combinations of two or more DNA repair targeted agents. Some caution is warranted in combination strategies that result in simultaneous deficiencies in more than one DNA repair pathway (whether endogenous or pharmacologic), as they may have variable effects on drug sensitivity or resistance depending on the specific pathway or drug (e.g., MMR deficiencies are associated with either resistance or sensitivity to different chemotherapeutics; ref. 85).

Studies are also coupling DNA repair targeted agents with other classes of targeted drugs, including MAPK or PI3K inhibitors or antiangiogenic agents. For instance, combinations of PARP inhibitors with VEGFR inhibitors (e.g., olaparib/cediranib; ref. 86) are advancing in clinical trials, and PI3K inhibitors appear to sensitize to PARP inhibitors in several preclinical studies (87, 88).

Finally, cells deficient in certain DDR pathways (especially MMR and proofreading DNA polymerase epsilon, and probably HRR) may exhibit greater responsiveness to immunotherapy due to increased neoantigens as a consequence of high mutation frequencies (89–91). In cells lacking such defects, adding DNA repair targeted agents could increase endogenous DNA damage and enhance responses to immunotherapy. However, DNA repair deficiencies may have other unforeseen immunologic consequences, such as impaired antigen presentation in MMR-deficient cells with mutations in a microsatellite that ablates beta2-microglobulin (92). Combinations of PARPi plus immunotherapy targeting PD-1/PD-L1 are entering clinical trials (Supplementary Table S1).

Despite optimism for DNA repair targeted agents, some caution is in order. Treatment with DNA repair inhibitors could increase mutation rates in malignant cells, leading to evolution of metastatic properties and/or drug resistance. Systemic DNA damage could increase the risk of secondary malignancies. For example, myelodysplastic syndrome risk may increase with platinum and PARPi (93).

In summary, due to the fundamental reliance of cancer cells upon DDR pathways, DNA repair targeted agents represent an exciting group of emerging therapeutics with potential to improve outcomes across a variety of cancer types. Identification and validation of accurate biomarkers of response and resistance to DNA repair targeted agents will improve patient selection and increase the clinical value of DNA repair targeted therapy.

Disclosure of Potential Conflicts of Interest

P.A. Konstantinopoulos is a consultant/advisory board member for Merck and Vertex Pharmaceuticals. U.A. Matulonis is a consultant/advisory board member for AstraZeneca, Clovis, Genentech/Roche, Immunogen, and Merck. E.M. Swisher is a consultant/advisory board member for IDEAYA Biosciences. No potential conflicts of interest were disclosed by the other author.

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Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial

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Summary

Background Poly(ADP-ribose) polymerase (PARP) inhibitors have activity in ovarian carcinomas with homologous recombination deficiency. Along with *BRCA1* and *BRCA2* (*BRCA*) mutations genomic loss of heterozygosity (LOH) might also represent homologous recombination deficiency. In ARIEL2, we assessed the ability of tumour genomic LOH, quantified with a next-generation sequencing assay, to predict response to rucaparib, an oral PARP inhibitor.

Methods ARIEL2 is an international, multicentre, two-part, phase 2, open-label study done at 49 hospitals and cancer centres in Australia, Canada, France, Spain, the UK, and the USA. In ARIEL2 Part 1, patients with recurrent, platinum-sensitive, high-grade ovarian carcinoma were classified into one of three predefined homologous recombination deficiency subgroups on the basis of tumour mutational analysis: *BRCA* mutant (deleterious germline or somatic), *BRCA* wild-type and LOH high (LOH high group), or *BRCA* wild-type and LOH low (LOH low group). We prespecified a cutoff of 14% or more genomic LOH for LOH high. Patients began treatment with oral rucaparib at 600 mg twice per day for continuous 28 day cycles until disease progression or any other reason for discontinuation. The primary endpoint was progression-free survival. All patients treated with at least one dose of rucaparib were included in the safety analyses and all treated patients who were classified were included in the primary endpoint analysis. This trial is registered with ClinicalTrials.gov, number NCT01891344. Enrolment into ARIEL2 Part 1 is complete, although an extension (Part 2) is ongoing.

Findings 256 patients were screened and 206 were enrolled between Oct 30, 2013, and Dec 19, 2014. At the data cutoff date (Jan 18, 2016), 204 patients had received rucaparib, with 28 patients remaining in the study. 192 patients could be classified into one of the three predefined homologous recombination deficiency subgroups: *BRCA* mutant (n=40), LOH high (n=82), or LOH low (n=70). Tumours from 12 patients were established as *BRCA* wild-type, but could not be classified for LOH, because of insufficient neoplastic nuclei in the sample. The median duration of treatment for the 204 patients was 5·7 months (IQR 2·8–10·1). 24 patients in the *BRCA* mutant subgroup, 56 patients in the LOH high subgroup, and 59 patients in the LOH low subgroup had disease progression or died. Median progression-free survival after rucaparib treatment was 12·8 months (95% CI 9·0–14·7) in the *BRCA* mutant subgroup, 5·7 months (5·3–7·6) in the LOH high subgroup, and 5·2 months (3·6–5·5) in the LOH low subgroup. Progression-free survival was significantly longer in the *BRCA* mutant (hazard ratio 0·27, 95% CI 0·16–0·44, p<0·0001) and LOH high (0·62, 0·42–0·90, p=0·011) subgroups compared with the LOH low subgroup. The most common grade 3 or worse treatment-emergent adverse events were anaemia or decreased haemoglobin (45 [22%] patients), and elevations in alanine aminotransferase or aspartate aminotransferase (25 [12%]). Common serious adverse events included small intestinal obstruction (10 [5%] of 204 patients), malignant neoplasm progression (10 [5%]), and anaemia (nine [4%]). Three patients died during the study (two because of disease progression and one because of sepsis and disease progression). No treatment-related deaths occurred.

Interpretation In patients with *BRCA* mutant or *BRCA* wild-type and LOH high platinum-sensitive ovarian carcinomas treated with rucaparib, progression-free survival was longer than in patients with BRCA wild-type LOH low carcinomas. Our results suggest that assessment of tumour LOH can be used to identify patients with *BRCA* wild-type platinum-sensitive ovarian cancers who might benefit from rucaparib. These results extend the potential usefulness of PARP inhibitors in the treatment setting beyond *BRCA* mutant tumours.

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Research in context

Evidence before this study

To identify other clinical trials of poly(ADP-ribose) polymerase (PARP) inhibitors for the treatment of ovarian cancer, we searched PubMed for articles published up to July 28, 2016, using the following search terms: ("PARP inhibitor" OR rucaparib OR olaparib OR niraparib OR veliparib OR talazoparib) AND (ovarian AND [cancer OR carcinoma]). Our search identified several clinical trials with results reporting antitumour activity and progression-free survival with PARP inhibitor monotherapy in patients with ovarian carcinoma with or without a *BRCA* mutation. Although the findings of some of these clinical studies suggested activity in patients without a *BRCA* mutation, no specific biomarkers were tested in a trial of a PARP inhibitor in patients with ovarian carcinoma with measurable disease. There is currently no optimum method to identify which *BRCA* wild-type cancers are most likely to respond to a PARP inhibitor.

Added value of this study

Our results show that a tumour-based, next-generation sequencing homologous recombination deficiency assay combining *BRCA* mutation status and percentage of genome-wide loss of heterozygosity (LOH) in the tumour could identify which patients with platinum-sensitive carcinomas without a germline *BRCA* mutation are most likely to respond to rucaparib treatment. Using our novel algorithm, we found that patients with a germline or somatic *BRCA* mutation or wild-type *BRCA* with high LOH had longer progression-free survival and more objective responses with rucaparib treatment than did patients with wild-type *BRCA* and low LOH. The findings of ARIEL2 Part 1 also showed that the mutation and methylation status of other homologous recombination-related genes, such as *RAD51C*, can be associated with high genomic LOH in *BRCA* wild-type tumours and with rucaparib response.

Implications of all the available evidence

PARP inhibitors have been shown to have activity in patients with a germline or somatic *BRCA* mutation; however, there are no proven predictive biomarkers of response to PARP inhibition in patients with a *BRCA* wild-type tumour. The results of ARIEL2 greatly extend the usefulness of PARP inhibitors as a treatment for cancer. Additionally, our data provide evidence that our LOH analysis is more sensitive than either mutational or methylation analyses for the identification of responders in this setting and should be assessed in other tumour types in which homologous recombination deficiency might be common.

Introduction

Ovarian cancer is the fifth leading cause of death due to cancer in women in both the USA and European Union.[1,2] Mutations in one allele of *BRCA1* or *BRCA2* (*BRCA*) accompanied by loss of the wild-type allele hinders homologous recombination-mediated DNA damage repair,[3] leading to loss or duplication of chromosomal regions, also known as genomic loss of heterozygosity (LOH).[4–6] Half of all high-grade serous ovarian carcinomas are estimated to have homologous recombination deficiency, with about 15% of carcinomas harbouring a germline *BRCA* mutation, 6% a somatic *BRCA* mutation, and 20% a mutation in, or epigenetic silencing of, another homologous recombination gene.[7,8] Even without an identifiable mutation in *BRCA* or other known homologous recombination gene, many high-grade serous ovarian carcinomas show *BRCA* mutant-like genomic signatures,[6,9] which could serve as a downstream marker of homologous recombination deficiency.

Poly(ADP-ribose) polymerase (PARP) enzymes are involved in DNA repair through activation of the base excision repair and alternative end-joining pathways and inhibition of the non-homologous end-joining pathway.[10,11] PARP inhibition in cells with homologous recombination deficiency is postulated to cause accumulation of unrepaired DNA double-strand breaks, ultimately leading to cell death.[10–12] Consequently, PARP inhibitors are selectively lethal in cells with homologous recombination deficiency.[10,11,13–18] In clinical trials, PARP inhibitors have shown antitumour activity and extended progression-free survival compared with placebo in patients with or without a *BRCA* mutation;[19–22] however, the optimal method for the identification of which *BRCA* wild-type cancers are most likely to respond to a PARP inhibitor is unknown.[20–23]

Results from a phase 1/2 study[24] of rucaparib, an oral PARP inhibitor, have shown efficacy and safety in women with relapsed, platinum-sensitive, high-grade ovarian carcinoma harbouring a germline *BRCA* mutation, with 22 (67%) of 33 patients achieving an objective response. The aim of ARIEL2 Part 1 was to identify molecular predictors of rucaparib sensitivity in patients with platinum-sensitive recurrent high-grade ovarian carcinoma, including tumours without a germline or somatic *BRCA* mutation.

Methods

Study design and participants

ARIEL2 is an international, multicentre, two-part, phase 2, open-label study designed to assess rucaparib sensitivity in three prospectively defined subgroups (appendix pp 6). The study protocol is available in the appendix. Data are presented for ARIEL2 Part 1, which has completed enrolment; an extension (Part 2) of ARIEL2, added through a protocol amendment (May 11, 2015), is ongoing and will be published separately.

Investigators at each site identified eligible patients according to recruitment strategies approved by each centre and offered them the chance to enrol. Patients were eligible to enrol in ARIEL2 Part 1 if they had

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high-grade serous or endometrioid ovarian, fallopian tube, or primary peritoneal carcinoma and had received at least one previous platinum therapy. Eligible patients were at least 18 years old, had not previously received a PARP inhibitor, had progressed 6 months or more after their most recent platinum-based treatment, had an Eastern Cooperative Oncology Group Performance Status of 0 to 1, and had disease that was measurable with the Response Evaluation Criteria In Solid Tumors version 1.1 (RECIST) and amenable to biopsy at trial entry. Patients were ineligible if they had an active second malignancy, central nervous system metastases, or had received anticancer therapy 14 days or fewer before receiving their first dose of rucaparib. Formalin-fixed paraffin-embedded archival and pretreatment tumour biopsies of adequate quality were required for each patient. A complete list of inclusion and exclusion criteria is provided in the appendix (pp 16–17).

The study was done at 49 hospitals and cancer centres in Australia, Canada, France, Spain, the UK, and the USA. ARIEL2 was approved by the institutional review board at each study site and was done in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines of the International Conference on Harmonisation. Patients provided written informed consent before participation.

Procedures

Patients were treated with oral rucaparib at 600 mg twice per day for continuous 28 day cycles until disease progression or any other reason for discontinuation. Supportive care (eg, antiemetics or analgesics for pain control) was permitted at the investigator's discretion. Dose reductions (in increments of 120 mg) were permitted if a patient had a grade 3 or worse adverse event. Treatment was discontinued if a dose interruption occurred for more than 14 consecutive days (longer dose interruptions were permitted with sponsor approval). Further details about dose modifications are shown in the appendix (p 2).

Tumour response was assessed by the investigators in line with RECIST, with CT scans at screening and every 8 weeks during treatment (and post-treatment for patients who discontinued for any reason other than disease progression). Assessments continued until confirmed disease progression, death, start of subsequent treatment, or loss to follow-up. Serum CA-125 measurements were taken at screening, day 1 of each cycle, the end of treatment, and when clinically indicated. Haematology, serum chemistry, and safety assessments were done at screening, day 1 and day 15 of cycle 1, and day 1 of any subsequent cycles. For pharmacokinetic analyses, a blood sample was taken on day 15 of cycle 1 and on day 1 of cycles 2, 3, and 4, before dosing with rucaparib and as close to 12 h after the last dose was taken as possible. Rucaparib pharmacokinetics were assessed with trough plasma concentrations (appendix pp 4–5). Adverse events were classified in accordance with the Medical Dictionary for Drug Regulatory Activities classification system version 18.1[25] and graded for severity in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03.[26]

At enrolment we used the Foundation Medicine T5 next-generation sequencing assay (Foundation Medicine, Cambridge, MA, USA)[27] to calculate the percentage of genomic LOH in archival and pretreatment biopsies.[27,28] We prespecified a cutoff of 14% or more to define LOH high, which was based on analysis of The Cancer Genome Atlas (TCGA) microarray and survival data for patients with ovarian carcinoma who had received platinum-based chemotherapy (appendix pp 2–5, 7, 18).[7] We classified patients into one of three predefined homologous recombination deficiency subgroups on the basis of this tumour analysis: *BRCA* mutant (deleterious germline or somatic), *BRCA* wild-type and LOH high (LOH high group), or *BRCA* wild-type and LOH low (LOH low group).

The tumour sequencing assay also identified mutations in homologous recombination genes other than *BRCA1* and *BRCA2* (appendix pp 3, 19). We assessed *BRCA1* and *RAD51C* promoter hypermethylation in tumours using a methylation-sensitive polymerase chain reaction (appendix p 4).[28,29] Mutations detected in tumour tissue were identified as germline or somatic by analysis of genomic DNA from blood by use of the BROCA-homologous recombination sequencing assay (University of Washington, Seattle, WA, USA).[30] For each patient, we used the most recently collected tumour specimen (ie, pretreatment biopsy if available or archival biopsy if not) to classify *BRCA* mutation, genomic LOH, and methylation status (appendix pp 4–5). Tumour tissue sequencing analyses were all done at the Foundation Medicine central laboratory (Cambridge, MA, USA).

Outcomes

In ARIEL2 Part 1, the primary endpoint was progression-free survival, defined as the time from the first dose of rucaparib to investigator-assessed disease progression or death from any cause. Secondary endpoints were the proportion of patients achieving an objective response (according to RECIST and Gynecological Cancer InterGroup [GCIG] CA-125 criteria),[31,32] duration of response (according to RECIST), safety, and pharmacokinetics. The proportion of patients achieving an objective response was defined as the proportion with a best response of complete or partial response. All RECIST and CA-125 responses were confirmed by a second assessment after at least 4 weeks. The combined proportion of patients achieving a RECIST or CA-125 objective response was assessed with GCIG combined RECIST and CA-125 criteria.[32] Duration of confirmed response (complete or partial response) was calculated from the initial date a response was detected to the first date of progressive disease. Tumour assessments were done by the investigators. Prior to study enrolment, each

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patient's LOH status was unknown, and investigators were not provided the results of the LOH analysis during the study. Investigators were not blinded to *BRCA* mutation status because patients could enrol with a known germline *BRCA* mutation, and information about a *BRCA* mutation detected upon analysis of tumour tissue during the study was provided to consenting patients and investigators.

Exploratory endpoints included comparison of LOH classification in archival and pretreatment biopsies and RECIST and CA-125 response in patients with a mutation in a non-*BRCA* homologous recombination gene.

Statistical analysis

After reviewing data from the TCGA, we estimated that 30% of patients eligible for ARIEL2 Part 1 (ie, those with platinum-sensitive ovarian cancer) would be classified in the *BRCA* mutant subgroup, 30–50% in the LOH high subgroup, and 20–40% in the LOH low subgroup. Thus, ARIEL2 Part 1 was designed to enrol at least 180 patients such that any of the three possible pairwise comparisons of subgroups would contain at least 100 patients, with each of the three comparisons resulting in 80% power at a two-sided 10% significance level to detect a difference in progression-free survival distributions (assuming the hazard ratio [HR] between two subgroups was 0·50). Comparisons between the *BRCA* mutant and LOH high subgroups were outside the scope of this study. The number of patients with a known deleterious germline *BRCA* mutation was capped at 15 to ensure enough patients with *BRCA* wild-type tumours were enrolled to test the hypothesis that LOH status in patients with *BRCA* wild-type tumours would be correlated with progression-free survival and objective response. Patients who were in the screening process when the target enrolment of 180 patients was reached were allowed to complete screening and enrol into the study if eligible.

All efficacy and safety analyses were done with the safety population, which included all patients who were

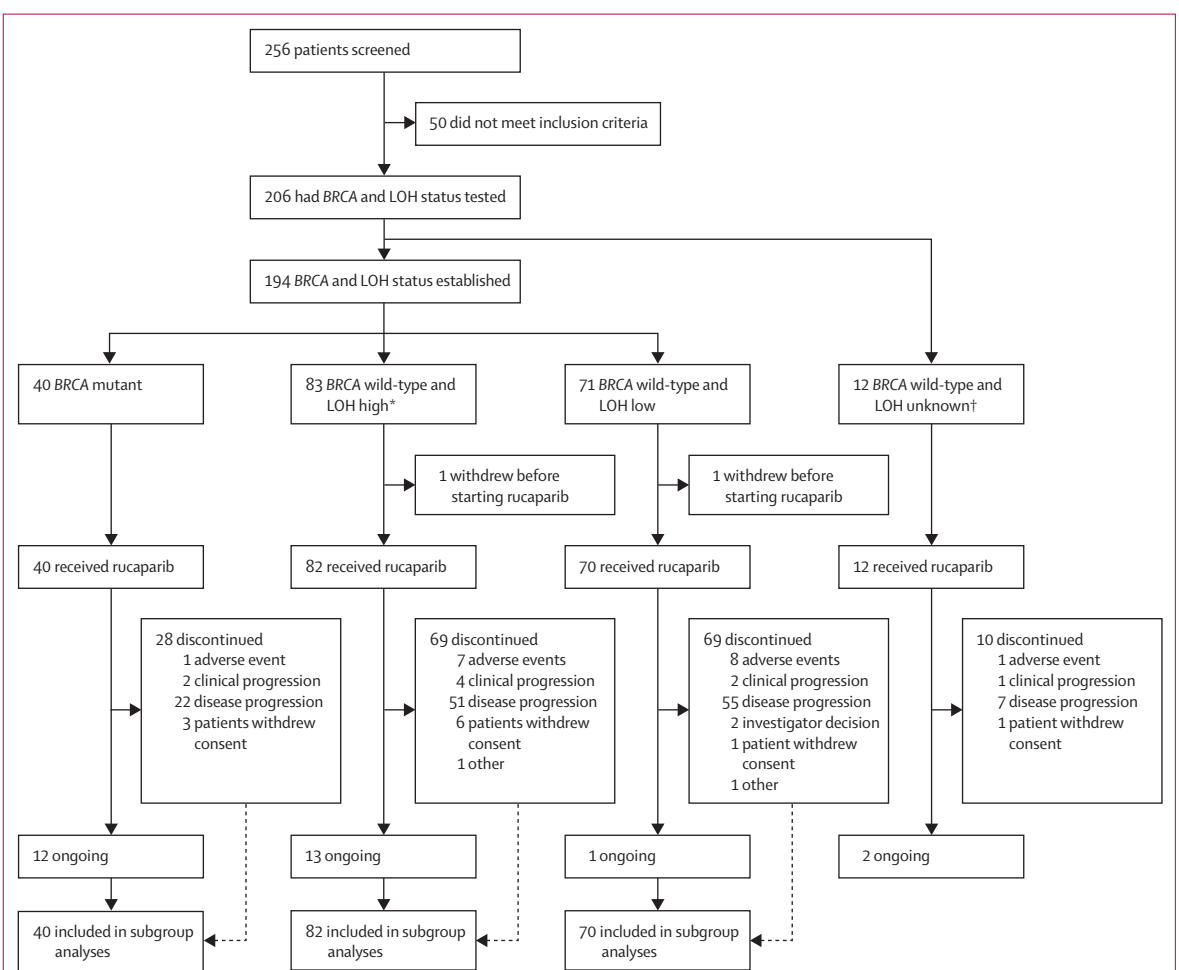


Figure 1: **Trial profile**

LOH=loss of heterozygosity. *Patients had genomic LOH ≥14%. †Sequencing of archival and pretreatment tumour samples from one patient did not pass quality check; therefore, the tumour cannot be definitively concluded to be *BRCA* wild-type.

treated with at least one dose of rucaparib. We analysed progression-free survival with Kaplan-Meier methods and a Cox proportional hazard model (two-sided test at the 5% significance level with 95% CI) to compare the *BRCA* mutant and LOH high subgroups with the LOH low subgroup. Patients without documented progression were censored as of their last tumour assessment. We analysed duration of response with Kaplan-Meier methods, with the log-rank test used to compare the distribution between subgroups. Patients with an ongoing response were censored as of their latest post-baseline scan. We used Clopper-Pearson methods to present proportions of patients achieving objective responses as percentages with 95% CIs and analysed differences between subgroups using a χ^2 test of proportions. We also did a post-hoc analysis of the best percentage change in the sum of all target lesions compared with baseline. We used SAS version 9.3 for the statistical analyses of progression-free survival, duration of response, objective response, and best percentage change in target lesions. We compared LOH classification in archival and pretreatment biopsies using Fisher's exact test. As an exploratory analysis, we also compared the sensitivity of different biomarkers (eg, genomic LOH, homologous recombination gene mutations, and methylation status) for the detection of RECIST response in patients with *BRCA* wild-type tumours using McNemar's test. We used R version 3.3.1 for the statistical analyses of comparison of LOH classification and sensitivity for the detection of response.

The principal investigators and sponsor personnel oversaw study conduct and reviewed risk–benefit every 6 months. ARIEL2 is registered with ClinicalTrials.gov, number NCT01891344.

Role of the funding source

The study was designed by the funder and a subgroup of investigators. Data presented herein were collected by the funder; the funder and all authors interpreted and analysed the data. Writing and editorial assistance were supported by the funder. EMS, KKL, HG, TCH, SG, LMal, JI, ARA, LR, MR, and IAM had access to the raw data. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

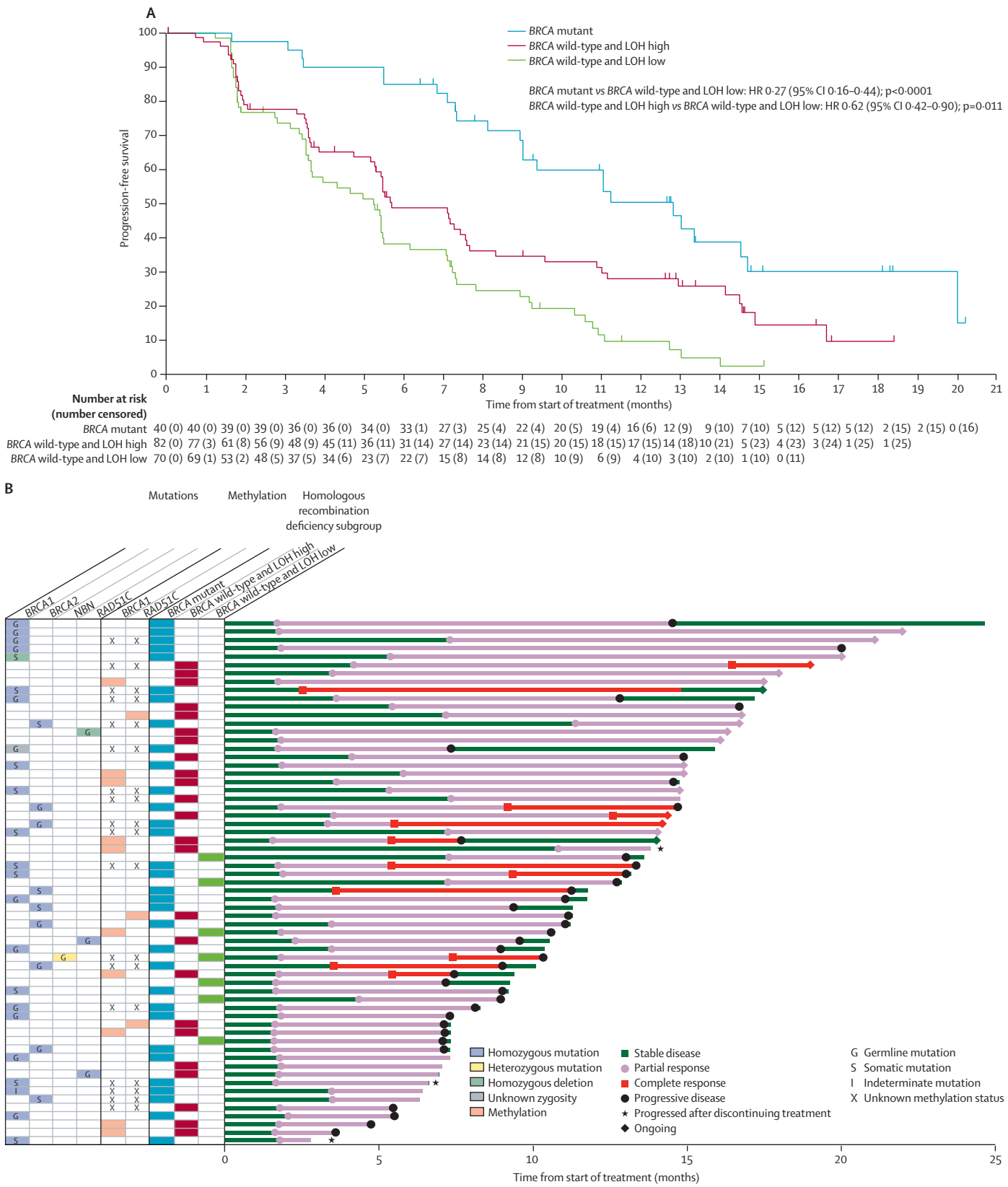
Results

Between Oct 30, 2013, and Dec 19, 2014, 256 patients were screened and 206 patients were enrolled into the trial (figure 1). At the data cutoff date (Jan 18, 2016), 204 patients had been treated with rucaparib, with 28 patients remaining on study medication. The median duration of treatment for the 204 patients was 5·7 months (IQR 2·8–10·1). 192 treated patients could be classified into one of the three predefined homologous recombination deficiency subgroups: *BRCA* mutant (n=40), LOH high (n=82), or LOH low (n=70). Tumours from 12 patients were established as *BRCA* wild-type, but could not be classified for LOH because of insufficient neoplastic nuclei (appendix p 8). Table 1 shows the demographic and disease characteristics of the enrolled patients. In view of the enrolment cap for known *BRCA* mutation carriers, only 20 (10%) of 204 patients were confirmed to have a germline *BRCA* mutation (14 had *BRCA1* mutations and six had *BRCA2* mutations) by use of the BROCA-homologous recombination assay. 19 (9%) other patients had a somatic *BRCA* mutation (14 had *BRCA1* mutations and five had *BRCA2* mutations) identified with tumour sequencing and the BROCA-homologous recombination assay. The germline or somatic status of one *BRCA1* mutation could not be established. 20 (10%) other patients had a somatic or germline mutation in another homologous recombination gene (appendix p 20). Of 165 tumours for which methylation analyses were completed, *BRCA1* promoter hypermethylation was detected in 21 (13%) tumours and *RAD51C* promoter hypermethylation was detected in four (2%) tumours. Methylation of *BRCA1* and *RAD51C* was only seen in tumours that did not harbour a germline or somatic mutation in *BRCA* or *RAD51C*.

	BRCA mutant (n=40)	*BRCA* wild-type and LOH high (n=82)	*BRCA* wild-type and LOH low (n=70)	*BRCA* wild-type and LOH unclassified (n=12)*
Age (years)	58·5 (53·5–67·5)	65·0 (58·0–71·0)	65·0 (55·0–72·0)	69·5 (63·0–77·0)
ECOG performance status				
0	26 (65%)	52 (63%)	47 (67%)	9 (75%)
1	14 (35%)	30 (37%)	23 (33%)	3 (25%)
Diagnosis†				
Epithelial ovarian cancer	38 (95%)	68 (83%)	49 (70%)	9 (75%)
Primary peritoneal cancer	1 (3%)	10 (12%)	12 (17%)	1 (8%)
Fallopian tube cancer	1 (3%)	4 (5%)	9 (13%)	2 (17%)
Histology				
Serous	39 (98%)	80 (98%)	66 (94%)	12 (100%)
Endometrioid	1 (3%)	1 (1%)	2 (3%)	0
Mixed	0	1 (1%)	2 (3%)	0
Previous treatment regimens				
Number of regimens	2 (1–2)	1 (1–2)	1 (1–2)	1 (1–1)
1	17 (43%)	44 (54%)	47 (67%)	10 (83%)
≥2	23 (58%)	38 (46%)	23 (33%)	2 (17%)
Number of platinum-based regimens	2 (1–2)	1 (1–2)	1 (1–2)	1 (1–1)
1	17 (43%)	45 (55%)	49 (70%)	10 (83%)
≥2	23 (58%)	37 (45%)	21 (30%)	2 (17%)
Progression-free interval after completion of platinum-based chemotherapy				
6 to <12 months	23 (58%)	37 (45%)	31 (44%)	5 (42%)
≥12 months	17 (43%)	45 (55%)	39 (56%)	7 (58%)

Data are median (IQR) or n (%). ECOG=Eastern Cooperative Oncology Group. LOH=loss of heterozygosity. *12 patients whose tumour specimens had sufficient nuclei to categorise as *BRCA* wild-type, but insufficient nuclei for genomic LOH analysis. †Diagnosis was unknown for one patient.

Table 1: **Demographic and disease characteristics by homologous recombination deficiency subgroup**


A
Progression-free survival
Time from start of treatment (months)
BRCA mutant
BRCA wild-type and LOH high
BRCA wild-type and LOH low
BRCA mutant vs BRCA wild-type and LOH low: HR 0·27 (95% CI 0·16–0·44); p<0·0001
BRCA wild-type and LOH high vs BRCA wild-type and LOH low: HR 0·62 (95% CI 0·42–0·90); p=0·011
Number at risk (number censored)
BRCA mutant 40 (0) 40 (0) 39 (0) 39 (0) 36 (0) 36 (0) 34 (0) 33 (1) 27 (3) 25 (4) 22 (4) 20 (5) 19 (4) 16 (6) 12 (9) 9 (10) 7 (10) 5 (12) 5 (12) 5 (12) 2 (15) 2 (15) 0 (16)
BRCA wild-type and LOH high 82 (0) 77 (3) 61 (8) 56 (9) 48 (9) 45 (11) 36 (11) 31 (14) 27 (14) 23 (14) 21 (15) 20 (15) 18 (15) 17 (15) 14 (18) 10 (21) 5 (23) 4 (23) 3 (24) 1 (25) 1 (25)
BRCA wild-type and LOH low 70 (0) 69 (1) 53 (2) 48 (5) 37 (5) 34 (6) 23 (7) 22 (7) 15 (8) 14 (8) 12 (8) 10 (9) 6 (9) 4 (10) 3 (10) 2 (10) 1 (10) 0 (11)
B
Mutations
Methylation
Homologous recombination deficiency subgroup
BRCA1
BRCA2
NBN
RAD51C
BRCA1
RAD51C
BRCA mutant
BRCA wild-type and LOH high
BRCA wild-type and LOH low
Time from start of treatment (months)
Homozygous mutation
Heterozygous mutation
Homozygous deletion
Unknown zygosity
Methylation
Stable disease
Partial response
Complete response
Progressive disease
Progressed after discontinuing treatment
Ongoing
G Germline mutation
S Somatic mutation
I Indeterminate mutation
X Unknown methylation status

24 patients in the *BRCA* mutant subgroup, 56 patients in the LOH high subgroup, and 59 patients in the LOH low subgroup had disease progression or died. Median progression-free survival after rucaparib treatment was 12·8 months (95% CI 9·0–14·7) in the *BRCA* mutant subgroup, 5·7 months (5·3–7·6) in the LOH high subgroup, and 5·2 months (3·6–5·5) in the LOH low subgroup (figure 2A). Progression-free survival was significantly longer in the *BRCA* mutant subgroup (HR 0·27, 95% CI 0·16–0·44, p<0·0001) and LOH high subgroup (HR 0·62, 0·42–0·90, p=0·011) than in the LOH low subgroup (figure 2A). 12 month progression-free survival was higher in the *BRCA* mutant subgroup (50%, 95% CI 33–65) and LOH high subgroup (28%, 18–39) than in the LOH low subgroup (10%, 4–19). The proportionality of hazards assumption was not violated (appendix pp 4–5, 15).

Confirmed objective RECIST responses are shown in table 2. The proportion of patients achieving RECIST responses was significantly higher in the *BRCA* mutant (p<0·0001) and LOH high (p=0·0033) subgroups than in the LOH low subgroup. The proportion of patients who achieved a response was similar irrespective of whether the *BRCA* mutation was germline or somatic or whether a patient had a *BRCA1* or *BRCA2* mutation (table 2). Confirmed combined RECIST and CA-125 responses were significantly more frequent in the *BRCA* mutant (p<0·0001) and LOH high (p=0·0018) subgroups than in the LOH low subgroup (table 2).

Median duration of response was longer in the *BRCA* mutant subgroup (9·2 months, 95% CI 6·4–12·9, p=0·013) and LOH high subgroup (10·8 months, 5·7–not reached, p=0·022) than in the LOH low subgroup (5·6 months, 4·6–8·5; appendix p 10). Pharmacokinetic data were obtained from 194 patients, including 40 from the *BRCA* mutant subgroup, 75 from the LOH high subgroup, 67 from the LOH low subgroup, and 12 with undetermined LOH status. Steady-state pharmacokinetics with rucaparib were achieved by cycle 1 day 15, with a mean trough plasma concentration of 2026 ng/mL (SD 1147; appendix pp 5, 9).

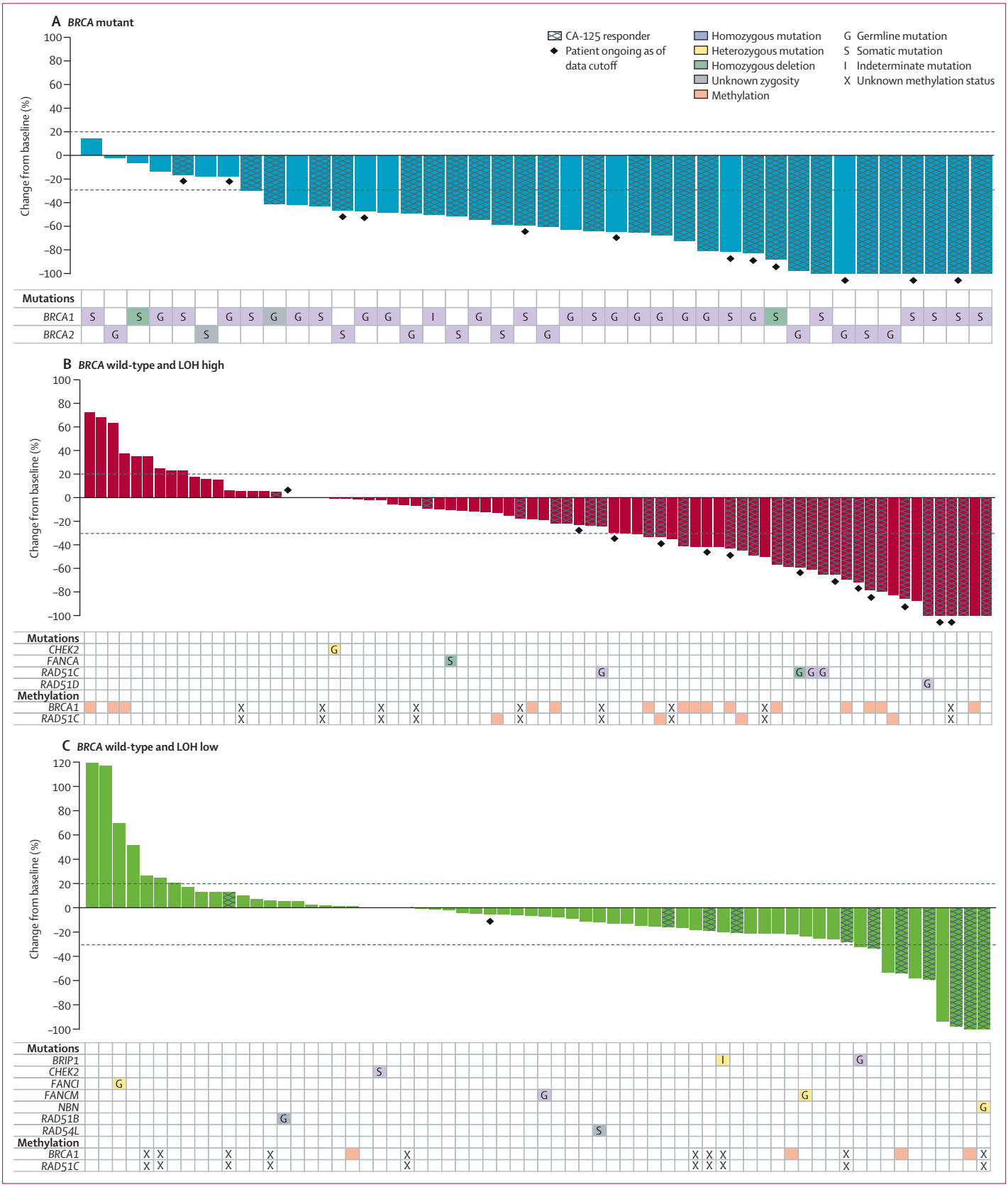
In an exploratory analysis, both RECIST and CA-125 responses were detected in patients with a mutation in a non-*BRCA* homologous recombination gene (eg, *ATM, NBN, RAD51C,* or *RAD51D*; appendix p 20). Confirmed RECIST responses were also detected in patients with tumours with *BRCA1* methylation and *RAD51C* methylation (figure 2B). Post-hoc analysis of the best percentage change in the sum of all target lesions by RECIST compared with baseline is shown for each patient according to molecular subgroup in figure 3.

In our exploratory analysis, among *BRCA* wild-type tumours (both LOH high and LOH low subgroups), genomic LOH was a more sensitive predictor of response (sensitivity 78%) than was mutation of other homologous recombination genes (sensitivity 11%; p<0·0001 by McNemar's test) and methylation of *BRCA1* or *RAD51C* (sensitivity 48%, p<0·021; appendix p 11). However, genomic LOH was not more sensitive than an analysis that combined both mutation in other homologous recombination genes and methylation (sensitivity 59%, p=0·13).

All 204 patients had at least one treatment-emergent adverse event (table 3). The most common grade 3 or worse treatment-emergent adverse events were anaemia or decreased haemoglobin (45 [22%] patients) and elevations in alanine aminotransferase or aspartate aminotransferase (25 [12%]); elevations in blood creatinine were only grade 1 or 2. One or more serious treatment-emergent adverse events were reported in 50 (25%) patients. Common serious adverse events included small intestinal obstruction (10 [5%] patients), malignant neoplasm progression (10 [5%] patients), and anaemia (nine [4%] patients; appendix p 21). 80 (39%) of all 204 treated patients needed a dose reduction, most commonly for anaemia (28 [14%] patients) and nausea (22 [11%] patients; appendix p 22). 19 (9%) patients discontinued treatment with an adverse event as the main reason; fatigue was the most common reason, occurring in six (3%) patients (appendix p 23). Three patients died during the study (two because of malignant neoplasm progression and one because of sepsis and malignant neoplasm progression). No treatment-related deaths were reported.

For 117 patients, LOH analyses were completed on paired archival and pretreatment tissue; our exploratory analysis showed that LOH classification was highly concordant between archival and pretreatment samples (r=0·86, p<0·0001; appendix p 12). Of 50 patients with an LOH low archival specimen, 17 (34%) had an LOH high pretreatment specimen. Of the 17 patients with a change in classification from LOH low to LOH high, five had a partial response. In contrast, we did not detect any cases in which the classification changed from LOH high to LOH low between the archival and the pretreatment tissue. Methylation of *BRCA1* was also highly concordant in 90 paired samples (p<0·0001; appendix p 12). Of 13 patients with *BRCA1* methylation in the archival specimen, four (31%) had an unmethylated pretreatment sample. Only one patient had methylation in the pretreatment biopsy but not in the archival biopsy.

Figure 2: **Progression-free survival and response duration**
(A) Kaplan-Meier estimates of progression-free survival in all patients who received at least one dose of rucaparib, stratified by homologous recombination deficiency subgroup. (B) Swimlane plot of duration of response in patients classified into the three predefined homologous recombination deficiency subgroups with confirmed partial or complete RECIST responses. Each bar represents an individual patient with the length corresponding to length of time on study drug. Tiles to the left of the plot show the homologous recombination deficiency subgroup of each patient and homologous recombination gene mutation type (colour coded by type) or methylation type identified in tumour or blood samples. Four patients (one with a complete response and three with a partial response) are not included in B because their archival or pretreatment biopsy could not be classified into a homologous recombination deficiency subgroup. HR=hazard ratio. LOH=loss of heterozygosity. RECIST=Response Evaluation Criteria In Solid Tumors version 1.1.


A BRCA mutant
CA-125 responder
Patient ongoing as of data cutoff
Homozygous mutation
Heterozygous mutation
Homozygous deletion
Unknown zygosity
Methylation
G Germline mutation
S Somatic mutation
I Indeterminate mutation
X Unknown methylation status
Change from baseline (%)
Mutations
BRCA1
BRCA2
B BRCA wild-type and LOH high
Change from baseline (%)
Mutations
CHEK2
FANCA
RAD51C
RAD51D
Methylation
BRCA1
RAD51C
C BRCA wild-type and LOH low
Change from baseline (%)
Mutations
BRIP1
CHEK2
FANCI
FANCM
NBN
RAD51B
RAD54L
Methylation
BRCA1
RAD51C

Discussion

The results of ARIEL2 Part 1 show the activity of rucaparib in patients with relapsed platinum-sensitive, high-grade ovarian carcinoma. Our data also support the ability of a homologous recombination deficiency signature identified by an algorithm combining the percentage of tumour genomic LOH with *BRCA* mutation status to identify patients who may benefit from rucaparib treatment. To our knowledge, ARIEL2 is the first study to prospectively use a tumour-based, next-generation sequencing homologous recombination deficiency assay that combines *BRCA* mutation status and the percentage of genome-wide LOH in a novel algorithm to predict sensitivity to a PARP inhibitor in women with relapsed ovarian carcinoma. In ARIEL2 Part 1, the three groups defined by *BRCA* and LOH analysis had distinct outcomes. The *BRCA* mutant subgroup had a significantly longer progression-free survival and a higher proportion of patients achieving RECIST responses than did the LOH low subgroup. The proportions of rucaparib-treated patients who achieved responses were similar between patients with a somatic or germline *BRCA* mutation and with a *BRCA1* or *BRCA2* mutation.

For patients with a *BRCA* wild-type carcinoma, the benefit of rucaparib treatment was higher for those with an LOH high carcinoma than for those with an LOH low carcinoma. Although the two *BRCA* wild-type subgroups had similar median progression-free survival, the hazard ratio for disease progression or death was significant between the two subgroups. Additionally, more patients achieved confirmed RECIST responses, more patients achieved confirmed RECIST and CA-125 responses, and patients had longer response durations in the LOH high subgroup than did patients in the LOH low subgroup. The median duration of response for the LOH high subgroup was similar to that of the *BRCA* mutant subgroup, with 13 (16%) of 82 LOH high patients and 12 (30%) of 40 patients with *BRCA* mutations still on treatment at the cutoff date, supporting the ability of the homologous recombination deficiency assay to identify patients without a *BRCA* mutation who might achieve a durable response with rucaparib treatment. A retrospective analysis of these data suggested that a refined cutoff of 16% or greater in the LOH high subgroup provided the optimum discrimination of progression-free survival, objective response, and duration of response in patients with *BRCA* wild-type ovarian carcinoma.[33]

	Confirmed objective responses by RECIST	Objective responses by combined RECIST and CA-125
BRCA mutant (n=40)	32 (80%, 64–91)	34 (85%, 70–94)
Germline mutation (n=20)	17 (85%, 62–97)	17 (85%, 62–97)
Somatic mutation (n=19)	14 (74%, 49–91)	16 (84%, 60–97)
Indeterminate (n=1)	1 (100%, 3–100)	1 (100%, 3–100)
BRCA1 mutation (n=29)	23 (79%, 60–92)	25 (86%, 68–96)
BRCA2 mutation (n=11)	9 (82%, 48–98)	9 (82%, 48–98)
PFI ≥6 to <12 months (n=23)	20 (87%, 66–97)	20 (87%, 66–97)
PFI ≥12 months (n=17)	12 (71%, 44–90)	14 (82%, 57–96)
BRCA wild-type and LOH high (n=82)	24 (29%, 20–40)	36 (44%, 33–55)
BRCA wild-type and LOH low (n=70)	7 (10%, 4–20)	14 (20%, 11–31)
BRCA wild-type and LOH not classified (n=12)	4 (33%, 10–65)	7 (58%, 28–85)

Data are n (%, 95% CI). Confidence intervals calculated using Clopper-Pearson method. CA-125=cancer antigen 125. LOH=loss of heterozygosity. PFI=progression-free interval following completion of platinum-based chemotherapy. RECIST=Response Evaluation Criteria In Solid Tumors version 1.1.

Table 2: **Objective response rates by homologous recombination deficiency subgroup**

Figure 3: **Best response in size of target lesions**

Best percentage change from baseline in sum of longest diameter of target lesions according to RECIST for patients with both baseline and postbaseline measurements in the (A) *BRCA* mutant subgroup, (B) *BRCA* wild-type and LOH high subgroup, and (C) *BRCA* wild-type and LOH low subgroup. Each bar represents percentage change from baseline in sum of the longest diameter of target lesions for an individual patient according to RECIST. Upper dotted lines represent the threshold for progressive disease (20% increase in the sum of the longest diameter of the target lesions) and lower dotted lines show the threshold for partial response (30% decrease in the sum of the longest diameter of the target lesions). Tables below plots show homologous recombination gene mutations (colour coded by type) and methylation identified in the tumour samples. CA-125=cancer antigen 125. LOH=loss of heterozygosity. RECIST=Response Evaluation Criteria In Solid Tumors version 1.1.

Comparison of the outcomes in ARIEL2 Part 1 with other studies investigating PARP inhibitors is difficult because of the ambiguity in how *BRCA* wild-type cancers have been defined historically. For example, in a previous study of patients with recurrent platinum-sensitive ovarian carcinoma,[34] median progression-free survival was 5·7 months and seven (32%) patients achieved objective responses following use of single-agent olaparib in a subgroup of 22 patients without a germline *BRCA* mutation. However, the *BRCA* mutation status of the tumour was unknown in half of the patients in that subgroup (11 of 22 patients).[34] Additionally, we are not aware of any studies that have prospectively investigated progression-free survival or objective responses following platinum therapy in patients with relapsed, *BRCA* wild-type ovarian carcinoma, which makes it difficult to compare the results from ARIEL2 Part 1 with an expected frequency of response to platinum therapy.

Our results add to the increasing body of evidence showing the potential of homologous recombination deficiency analysis to identify patients who will benefit from PARP inhibitor treatment. Other biomarkers for homologous recombination deficiency have been assessed in previous studies,[4,35,36] for example, through retrospective analysis of *BRCA* mutations in ovarian carcinoma[21] or prospective identification of homozygous deletions or mutations through next-generation sequencing in prostate cancer.[22] Additionally, the NOVA

	Grade 1–2	Grade 3	Grade 4	Grade 5
Nausea	154 (75%)	9 (4%)	0	0
Asthenia or fatigue	141 (69%)	18 (9%)	0	0
Constipation	91 (45%)	3 (1%)	0	0
Vomiting	85 (42%)	4 (2%)	0	0
Dysgeusia	87 (43%)	0	0	0
Alanine aminotransferase or aspartate aminotransferase increased*	61 (30%)	24 (12%)	1 (<1%)	0
Decreased appetite	80 (39%)	4 (2%)	0	0
Anaemia; decreased haemoglobin	29 (14%)	43 (21%)	2 (1%)	0
Diarrhoea	61 (30%)	7 (3%)	0	0
Abdominal pain	56 (27%)	5 (2%)	0	0
Dyspnoea	46 (23%)	1 (<1%)	0	0
Abdominal distension	43 (21%)	0	0	0
Dizziness	37 (18%)	1 (<1%)	0	0
Urinary tract infection	33 (16%)	4 (2%)	0	0
Blood creatinine increased	34 (17%)	0	0	0
Headache	34 (17%)	0	0	0
Cough	33 (16%)	0	0	0
Back pain	30 (15%)	2 (1%)	0	0
Thrombocytopenia; platelet count decreased	25 (12%)	5 (2%)	0	0
Photosensitivity reaction	27 (13%)	0	0	0
Neutropenia; neutrophil count decreased	10 (5%)	9 (4%)	7 (3%)	0
Insomnia	25 (12%)	0	0	0
Pyrexia	24 (12%)	1 (<1%)	0	0
Abdominal pain (upper)	22 (11%)	0	0	0
Oedema peripheral	22 (11%)	0	0	0
Alopecia	21 (10%)	0	0	0
Stomatitis	20 (10%)	1 (<1%)	0	0
Upper respiratory tract infection	21 (10%)	0	0	0
Blood alkaline phosphatase increased	16 (8%)	3 (1%)	0	0
Dyspepsia	18 (9%)	1 (<1%)	0	0
Pain in extremity	17 (8%)	1 (<1%)	0	0
Weight decreased	16 (8%)	1 (<1%)	0	0
Dehydration	10 (5%)	6 (3%)	0	0
Myalgia	15 (7%)	1 (<1%)	0	0
Ascites	7 (3%)	7 (3%)	0	0
Blood cholesterol increased	11 (5%)	2 (1%)	0	0
Hypokalaemia	7 (3%)	5 (2%)	0	0
White blood cell count decreased	11 (5%)	1 (<1%)	0	0
Small intestinal obstruction	1 (<1%)	10 (5%)	0	0
Hydronephrosis	8 (4%)	2 (1%)	0	0
Malignant neoplasm progression	0	8 (4%)	1 (<1%)	3 (1%)
Blood bilirubin increased	7 (3%)	1 (<1%)	1 (<1%)	0
Mucosal inflammation	7 (3%)	1 (<1%)	0	0
Hypotension	5 (2%)	2 (1%)	0	0
Acute kidney injury	1 (<1%)	5 (2%)	0	0
Bronchitis	5 (2%)	1 (<1%)	0	0
Gamma-glutamyltransferase increased	1 (<1%)	4 (2%)	1 (<1%)	0
Hypercholesterolaemia	5 (2%)	1 (<1%)	0	0
Hyperglycaemia	4 (2%)	2 (1%)	0	0
Hypophosphataemia	1 (<1%)	5 (2%)	0	0
Rectal haemorrhage	5 (2%)	1 (<1%)	0	0
Fall	4 (2%)	1 (<1%)	0	0
Hyponatraemia	1 (<1%)	4 (2%)	0	0
Transaminases increased	2 (1%)	3 (1%)	0	0
Malaise	3 (1%)	1 (<1%)	0	0
Sepsis	0	1 (<1%)	2 (1%)	1 (<1%)
Leucopenia	2 (1%)	1 (<1%)	0	0
Presyncope	2 (1%)	1 (<1%)	0	0
Pulmonary embolism	1 (<1%)	2 (1%)	0	0
Syncope	0	3 (1%)	0	0
Food poisoning	1 (<1%)	1 (<1%)	0	0
Hyperbilirubinaemia	1 (<1%)	1 (<1%)	0	0
Lymphocyte count decreased	1 (<1%)	0	1 (1%)	0
Lymphoedema	1 (<1%)	1 (<1%)	0	0
Tachycardia	1 (<1%)	1 (<1%)	0	0
Pneumonia	0	2 (1%)	0	0
Agitation	0	1 (<1%)	0	0
Bile duct obstruction	0	1 (<1%)	0	0
Cataract	0	1 (<1%)	0	0
Dyspareunia	0	1 (<1%)	0	0
Empyema	0	1 (<1%)	0	0
Granulocytopenia	0	1 (<1%)	0	0
Hypermagnesaemia	0	1 (<1%)	0	0
Intestinal obstruction	0	1 (<1%)	0	0
Liver function test abnormal	0	1 (<1%)	0	0
Lymphangitis	0	1 (<1%)	0	0
Mental status changes	0	1 (<1%)	0	0
Peritonitis	0	1 (<1%)	0	0
Febrile neutropenia	0	0	1 (<1%)	0
Granulocyte count decreased	0	0	1 (<1%)	0
Intestinal perforation	0	0	1 (<1%)	0
Large intestinal obstruction	0	0	1 (<1%)	0
Long QT syndrome congenital	0	0	1 (<1%)	0
Acute myeloid leukaemia or myelodysplastic syndrome	0	0	0	0

(Table 3 continues in next column)

(Continued from previous column)

Data are n (%) in the safety population (n=204). *Elevations were transient, self-limiting, and not associated with other signs of liver toxicity.

Table 3: **Treatment-emergent adverse events**

trial (NCT01847274) prospectively tested a homologous recombination deficiency-based assay in a trial of niraparib as maintenance therapy in patients with platinum-sensitive ovarian cancer.[37] However, to our knowledge, ARIEL2 is the only study to prospectively assess a homologous recombination deficiency assay in patients with ovarian cancer who have measurable disease treated with a PARP inhibitor, thereby testing the assay as a biomarker for PARP inhibitor response. Other prospective trials in ovarian cancer are assessing homologous recombination deficiency assays in the maintenance setting following platinum therapy (eg, NOVA and ARIEL3 [NCT01968213]).

Our results also suggest that, in platinum-sensitive ovarian carcinomas, a mutation in a homologous recombination gene other than *BRCA1* or *BRCA2* (eg, *RAD51C*) or promoter hypermethylation of *BRCA1* or *RAD51C* can be associated with high genomic LOH and rucaparib response. However, not all homologous recombination gene mutations were associated with an LOH high genotype. Although the LOH analysis was more sensitive in the identification of responders in *BRCA* wild-type ovarian carcinomas than were either mutational or methylation analyses, LOH analysis was not more sensitive than mutation and methylation analyses combined. The high correlation of genomic LOH in archival and pretreatment biopsies suggests that either source can be used to predict response to rucaparib in this population of patients. However, a subset of patients whose archival tumour samples were defined as having low genomic LOH had increased genomic LOH in matched pretreatment tumour biopsies. This observation meant that recent biopsies had higher predictive sensitivity than did archival biopsies. Even in this platinum-sensitive population, loss of *BRCA1* methylation between the archival and pretreatment biopsy was detected in 31% of tumours. Data from patients with ovarian cancer with acquired chemotherapy resistance have shown that loss of *BRCA1* methylation could serve as a mechanism of therapeutic resistance.[38] Given that the homologous recombination deficiency status within a tumour might change over time, we recommend testing of the most recently collected tumour biopsy.

In ARIEL2, treatment-emergent adverse events were frequent and led to dose reductions in 39% of patients; however, only 9% of patients withdrew from the study as a result of a treatment-emergent adverse events. As with studies of other PARP inhibitors, treatment-emergent anaemia or decreased haemoglobin was the most common grade 3 adverse event. Anaemia was managed through transfusions and dose reductions. Alanine and aspartate aminotransferase levels increased after use of rucaparib; however, these increases were asymptomatic, reversible, and rarely associated with increased bilirubin levels. Patients with elevated alanine and aspartate aminotransferase levels were able to continue rucaparib treatment without dose reduction, and these elevations normalised over time.

Mild-to-moderate elevations in creatinine were also reported within the first few weeks following initiation of rucaparib treatment. Veliparib, another PARP inhibitor, has been reported to inhibit drug transporters expressed in the liver (MATE1) and kidneys (OCT2, MATE1, and MATE2-K).[39] Similarly, results from in-vitro studies have shown that rucaparib inhibits MATE1 and MATE2-K transporters, which have a role in the renal secretion of creatinine. Thus, inhibition of these transporters might be responsible for the increases in blood creatinine noted following rucaparib treatment. On the basis of this mechanism, elevations in serum creatinine should be assessed in conjunction with other laboratory parameters to assess renal function.

Our study had several limitations. Although ARIEL2 Part 1 identified a biomarker that seems to be predictive, it is possible that the homologous recombination deficiency assay is only prognostic; therefore, the predictive ability of the biomarker will need to be confirmed in the setting of a larger randomised study. Indeed, the refined LOH high cutoff of 16% or higher that was identified retrospectively in ARIEL2 Part 1[33] is being prospectively applied in the randomised, phase 3 ARIEL3 trial, which aims to assess progression-free survival and overall survival with rucaparib as maintenance therapy following platinum-based chemotherapy for patients with platinum-sensitive, recurrent ovarian carcinoma. The randomised design of ARIEL3 will enable confirmation of genomic LOH as a predictive biomarker. Additionally, it is not known whether the findings in ARIEL2 Part 1 will extend to patients whose disease is resistant or refractory to platinum therapy. Hence, the homologous recombination deficiency assay is also being prospectively tested in an extension (Part 2) of ARIEL2, which is investigating rucaparib in patients with carcinomas that are platinum-sensitive, platinum-resistant, or platinum-refractory; who have received at least three but not more than four prior chemotherapies; and have had a treatment-free interval of more than 6 months following first-line chemotherapy. The primary endpoint of ARIEL2 Part 2 is the proportion of patients achieving objective responses; progression-free survival and overall survival are key secondary endpoints. Additional studies should assess whether the homologous recombination deficiency assay developed in ARIEL2 predicts sensitivity to rucaparib and other PARP inhibitors in patients with other cancer types, including non-serous ovarian cancer, and gastric, pancreatic, prostate, or breast cancers.[9,22,40–42]

Contributors

EMS, CLS, HG, SHK, ARA, LR, MR, and IAM were involved in the study conception. EMS, KKL, HG, TCH, LMal, JI, ARA, LR, MR, and IAM were involved in the study design. EMS, RLC, RSK, JDB, SHK, and IAM acquired funding. EMS, KKL, and IAM were involved in the protocol development and co-wrote the first draft of the manuscript.

EMS, AMO, CLS, GEK, RLC, AVT, DMO, RSK, LMa, KMB-M, JDB, JMC, AO, IR-C, AF, AL, and IAM treated patients. EMS, KKL, AMO, CLS, GEK, RLC, AVT, DMO, RSK, LMal, KMB-M, JDB, JMC, AO, IR-C, MIH, SHK, AF, AL, and IAM acquired data. EMS, KKL, CLS, HG, JS, SHK, TCH, SG, LMal, JI, ARA, LR, RY, MR, and IAM interpreted the data. EM contributed to sample acquisition and management. MIH analysed data. All authors contributed to manuscript revisions and approved the final draft for submission.

Declaration of interests

KKL, HG, EM, TCH, SG, LMal, JI, LR, and MR are employees of Clovis Oncology; ARA was employed at Clovis Oncology at the time of the study and owns stock in the company. CLS's institution received in kind research support for parallel laboratory work using rucaparib. JS is a current employee and RY was an employee of Foundation Medicine, the developer of the homologous recombination deficiency assay used in ARIEL2. RLC reports grants from AstraZeneca, Genentech (Roche), Janssen, OncoMed, Millennium, Esperance, and AbbVie. AVT has served on an advisory board for and received grants from AstraZeneca. DMO has received research funding from Clovis Oncology; institutional research support from Amgen, VentiRx, Regeneron, Immunogen, Array Biopharma, Janssen R&D, Clovis Oncology, EMD Serono, Ergomed, Ajinomoto, and Genentech (Roche); and has served on a steering committee or advisory boards for Amgen, AstraZeneca, Janssen, Clovis Oncology, Genentech (Roche), and Eisai. During the conduct of the study, RSK served on an advisory board for Clovis Oncology. KMB-M served on advisory boards for Clovis Oncology and AstraZeneca. JDB has been advisor for and owns stock in Inivata, has served on a speakers' bureau for AstraZeneca, has received non-financial support from Clovis Oncology and Aprea AB, and has a pending patent for a diagnostic method of relevance to the current work. AO has served on advisory boards for Roche, AstraZeneca, Pharmamar, and Clovis. IR-C has served on an advisory board for AstraZeneca, Pharmamar, and Roche. SHK has a patent for a diagnostic method of relevance to the current work. AL has served on an advisory board for Clovis, Pfizer, and Pharmamar, and reports institutional research grant support from Gamamabs and Merus. IAM has served on advisory boards for Clovis Oncology and AstraZeneca. All other authors declare no competing interests.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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ONLINE SUPPLEMENTARY APPENDIX

Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial

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This appendix has been provided by the authors to give readers additional information about their work.

TABLE OF CONTENTS

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SUPPLEMENTAL METHODS

Dose modification criteria

Treatment with rucaparib was held and a dose reduction was considered or implemented if any of the following were observed: grade 3 or 4 haematologic toxicity; or grade 3 or 4 nonhaematologic toxicity (except for alopecia, nausea, vomiting, or diarrhoea adequately controlled with systemic antiemetic/antidiarrhoeal medication administered in standard doses according to the study centre routines). Rucaparib was not required to be held for grade 3 elevations of alanine/aspartate transaminase levels if not accompanied by other signs of liver dysfunction.

At the discretion of the investigator, rucaparib may have been held and/or the dose reduced for grade 2 toxicity not adequately controlled by concomitant medications and/or supportive care.

Treatment with rucaparib was held until the toxicity resolved to less than grade 2. If treatment resumed at the same dose and the patient experienced the same toxicity, the dose was reduced following resolution of the event to less than grade 2. If the patient continued to experience toxicity, additional dose reduction steps were permitted. If a patient continued to experience toxicity despite multiple dose reduction steps, or if dosing with rucaparib was interrupted for >14 consecutive days because of toxicity, treatment was discontinued, unless otherwise agreed between the investigator and the sponsor.

The Cancer Genome Atlas (TCGA) high-grade serous ovarian carcinoma analysis

The TCGA high-grade serous ovarian carcinoma study profiled ovarian carcinomas using the Affymetrix single nucleotide polymorphism (SNP) 6·0 arrays (Santa Clara, CA, USA) and deposited the patients' overall survival following platinum-based (and other standard of care) therapies. Affymetrix SNP 6·0 array intensity data (.CEL files) were downloaded from the controlled access TCGA database (https://tcga-data.nci.nih.gov/tcga/tcgaDownload.jsp, 2010-06-05 version). SNP genotype calls (.CHP files) were generated from the array intensity data using the Birdseed v2 algorithm with the default confidence threshold of 0·1 in the Affymetrix Genotyping Console. For loss of heterozygosity (LOH) inference, 2998 SNPs on the Affymetrix SNP 6·0 array were selected based on genome coverage and high heterozygous allele frequencies in the HapMap western

European population. LOH regions were inferred using unpaired analysis with Hidden Markov Model as previously described.[1] Default parameters were used for the unpaired analysis: expected genotype error rate of 0·01 and heterozygous frequency of 0·3. For each tumour, the percent genomic LOH was calculated as 100 times the total length of nonexcluded LOH regions divided by the total length of nonexcluded regions of the genome as described above.

Overall survival was analysed using the Kaplan-Meier methodology, and the estimated median and log-rank p value comparing high versus low percent genomic LOH was performed. Cox proportional hazards models were used to calculate the hazard ratios (HRs) and p values. Multivariate analysis encompassed genomic LOH status, *BRCA* mutation status, and residual postsurgical tumour burden as covariates.

Next generation sequencing (NGS)-based homologous recombination deficiency (HRD) assay

Formalin-fixed paraffin-embedded (FFPE) tumour specimens from the ARIEL2 study were profiled using Foundation Medicine's NGS-based T5a assay (Cambridge, MA, USA), which sequences 287 cancer-related genes and more than 3500 genome-wide SNPs and has been analytically validated to accurately detect all classes of genomic alterations.[2] Briefly, 50 to 200 ng of extracted DNA from FFPE tumour specimens was used for whole-genome shotgun library construction and hybridisation-based capture of all target regions. Using the Illumina® HiSeqTM 2500 platform (San Diego, CA, USA), hybrid-capture-selected libraries were sequenced to high uniform depth (targeting >500× coverage with >99% of exons at coverage >100×). NGS analysis could not be performed on 22% of pretreatment specimens primarily because of inadequate tumour cells (eg, <20% tumour nuclei) in the biopsy tissues (figure S3).

Sequence data were processed using a customised analysis pipeline designed to accurately detect base substitutions, short insertions/deletions, and homozygous gene deletions in *BRCA1/2* and other homologous recombination genes. Tumours with protein-truncating and splice-site mutations (±2 bp of exon starts/ends) in known or putative tumour suppressor genes were classified as potentially deleterious. Protein-truncating mutations were identified by the presence of a premature stop codon, with the exception of amino acids 3′ of codon K3226 in *BRCA2,* which are not deleterious.[3] In addition, *BRCA* mutation classification included *BRCA1/2* missense mutations known to be deleterious based on the Breast Cancer Information Core database,[4] although no deleterious missense mutations were identified in ARIEL2. Mutations were determined to be germline by sequencing of DNA extracted from blood using the NGS-based BROCA-HR test (University of Washington, Seattle, WA, USA) as previously described.[5]

Percent genomic LOH

To compute the percent genomic LOH for each tumour, LOH segments were inferred across the 22 autosomal chromosomes using the genome-wide aneuploidy/copy number profile and minor allele frequencies of the more than 3500 polymorphic SNPs sequenced in the Foundation Medicine's NGS-based T5a assay. Briefly, a comparative genomic hybridisation (ie, log-ratio profile of the sample) was obtained from the NGS sequencing data by normalising the sequence coverage obtained at all exons and genome-wide SNPs against a process-matched normal control. This profile was segmented and interpreted using allele frequencies of sequenced SNPs to estimate copy number (C_i) and minor allele count (M_i) at each segment (i). A segment was determined to have LOH if $C_i \neq 0$ and $M_i = 0$. Low tumour content or low aneuploidy were the most common reasons for failure to pass the quality control to perform genomic LOH inference.

Two types of LOH segments were excluded from the calculation of percent genomic LOH: (1) LOH segments spanning ≥90% of a whole chromosome or chromosome arm, as these LOH events usually arise through non-HRD mechanisms (eg, mitotic nondisjunction[6]), and (2) regions in which LOH inference was ambiguous.

For each tumour, the percent genomic LOH was computed as 100 times the total length of nonexcluded LOH regions (x_i) divided by the total length of nonexcluded regions of the genome. In equation form:

$$Percent\ genomic\ LOH = 100 \times \frac{\sum_i x_i}{L_{genome} - L_{exclusions}}$$

Where

x_i: Length of eligible LOH at segment i

L_{genome}: Total length of genome with SNP coverage, which is $2{\cdot}78\times10^9$ base pairs

$L_{exclusions}$: Total length of genome excluded for LOH analysis

Methylation assays

Five- to 10-micron sections of FFPE tissue were deparaffinised, rehydrated, and digested with Proteinase K (Zymo Research, Irvine, CA, USA) overnight. Bisulfite conversion of 10 µL of supernatant was performed in duplicate for each sample using the EZ DNA Methylation-Direct™ kit (Zymo Research). Following bisulfite conversion, the samples underwent desulphonation and cleanup; 2 µL of bisulfite-converted DNA were evaluated with methylation-specific polymerase chain reaction (PCR) for *BRCA1* as previously described.[7] Methylation-specific PCR for *RAD51C* was performed with newly designed primers. Primer sequences for the methylated reaction were 5′-TGTAAGGTTCGGAGTTTCGTGC-3′ (sense) and 5′-TCGCTAAAACGTACGACGTAACG-3′ (antisense) and for the unmethylated reaction 5′-GTGTAAAGTTGTAAGGTTTGGAGTTTTGTGTG-3′ (sense) and 5′-CACACACCCTCACTAAAACATACAACATAACA-3′ (antisense). The unmethylated product is 103 nucleotides and the methylated product 85 nucleotides. Positive controls for bisulfite conversion and for methylation were in vitro methylated DNA.

Validation of the *RAD51C* primers to accurately determine level of methylation had previously been undertaken on tumour tissue from a patient-derived xenograft with low *RAD51C* protein expression on Western blot and low mRNA expression on reverse transcriptase quantitative PCR (data not shown). Cloning of the bisulfite-altered DNA and Sanger sequencing from that tumour created methylation maps of single DNA molecules, demonstrating that the 16 CpG islands identified by the *RAD51C* methylation primers were centred in the most heavily methylated region.

Analysis of predictive utility for rucaparib response

Responders consisted of patients with complete or partial response, and nonresponders consisted of patients with stable disease and progressive disease using Response Evaluation Criteria In Solid Tumors version 1·1 (RECIST). For the receiver operating characteristic (ROC) analysis of the percent genomic LOH, the p value is the probability that the observed area under the curve (AUC) is found when the null hypothesis is AUC=0·5.

Pharmacokinetic evaluations

Trough plasma concentrations of rucaparib were determined using blood samples (4 mL) taken on day 15 of cycle 1 and on day 1 of cycles 2, 3, and 4, prior to dosing with rucaparib. A validated high-performance liquid chromatography-tandem mass spectrometry method with a calibration range of 5 to 10000 ng/mL was used.

Verification of the proportional hazards assumption

Proportionality was tested using SAS® Version 9.3 to ensure that the estimated survival function did not change over time (ie, the curves for the HRD subgroups did not cross) and that the plot of the log of the cumulative hazard for each subgroup resulted in parallel curves.

SUPPLEMENTAL RESULTS

Prespecification of percent genomic LOH cutoff

TCGA high-grade serous ovarian carcinoma study suggested that one approach to identify potential poly(ADP-ribose) polymerase (PARP) inhibitor–sensitive *BRCA*–wild-type patients may be to detect genetic and epigenetic alterations within genes in the homologous recombination repair pathway.[8] Since platinum sensitivity is an approximate surrogate marker for PARP inhibitor sensitivity,[9,10] we analysed overall survival following platinum-based chemotherapies in the TCGA dataset.

The cutoff for percent genomic LOH that separates high genomic LOH (LOH-high) from low genomic LOH (LOH-low) cancers was determined by analysing the TCGA high-grade serous ovarian carcinoma dataset.[8] We tested a wide range of genomic LOH cutoffs (8% to 20%) and found that optimal separation of overall survival curves between patients with LOH-high versus LOH-low high-grade ovarian carcinoma centred at a cutoff of 14% (HR, 0·62, p=0·0047; figure S2). Furthermore, genomic LOH was found to be an independent predictor of overall survival based on multivariate analysis, with *BRCA* mutation status and residual postsurgical tumour burden as covariates (table S2). Thus, we prespecified 14% as the percent genomic LOH cutoff for prospective testing in the ARIEL2 study.

NGS of archival and matched screening biopsies from ARIEL2

To identify genetic alterations and assess genomic LOH in ARIEL2, we performed a targeted NGS-based assay of 198 archival tumours and 152 pre-rucaparib treatment (pretreatment) biopsies from 206enrolled patients with high-grade ovarian carcinoma (figure S3). A total of 145 matched pairs of FFPE archival and pretreatment biopsies were

sequenced. The median time between archival and pretreatment biopsy was 2·7 years. We hypothesised that pretreatment biopsies would be more representative of the current state of tumour genetics and genomic scarring phenotype compared with archival specimens based on reported temporal heterogeneity in high-grade ovarian carcinoma.[11] Therefore, we prespecified that for each patient the most recently collected cancer specimen (ie, pretreatment biopsy if available, or archival tumour if not) would be used to determine the *BRCA* mutation status and genomic LOH level. Genomic LOH analysis was completed for 173 archival and 136 pretreatment tumour samples (117 matched pairs) from 194 patients.

Predictive utility of the percent genomic LOH

To test the overall predictive utility of the percent genomic LOH in order to identify rucaparib responders, ROC analysis was performed using pretreatment (if available) and archival samples from ARIEL2. ROC analysis indicated genomic LOH was highly predictive of RECIST responders (including unconfirmed responders) to rucaparib in all tumour evaluable patients, with an AUC of 0·75 ($p<0·0001$; figure S8A). Furthermore, after excluding *BRCA*-mutant carcinomas, genomic LOH remained significantly predictive of rucaparib response (AUC, 0·68; $p=0·0026$; figure S8B,C), indicating genomic LOH could be used to identify rucaparib responders in *BRCA*–wild-type patients.

Additional molecular characteristics of HRD subgroups

Specific genetic alterations were enriched in the different HRD subgroups (figure S9). For example, both *BRCA*-mutant and *BRCA*–wild-type/LOH-high subgroups had significantly higher frequencies of *MYC*-amplified tumours than the *BRCA*–wild-type/LOH-low subgroup ($p=0·0036$ and $p=0·029$), highlighting the molecular similarity between *BRCA*-mutant and *BRCA*–wild-type/LOH-high high-grade ovarian cancer. Conversely, *BRCA*-mutant and *BRCA*–wild-type/LOH-high subgroups had significantly lower frequencies of *CCNE1* amplification than the *BRCA*–wild-type/LOH-low subgroup ($p=0·00014$ and $p=0·031$), consistent with the reported synthetic lethality effect between *CCNE1* amplification and *BRCA1* loss.[12]

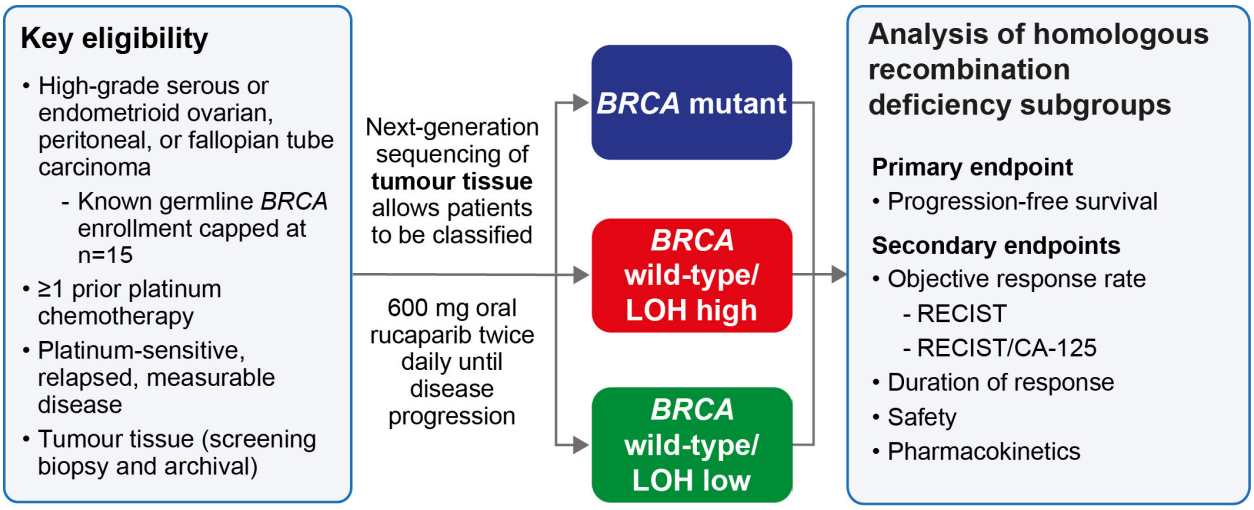
Proportional hazards assumption analysis

The estimated survival curves for the HRD subgroups did not cross (manuscript figure 2) and the plot of the log of the cumulative hazard for each subgroup resulted in parallel curves (supplementary figure S10), indicating that there is no violation of the Cox proportional hazards assumption.

Steady state rucaparib concentration

Pharmacokinetic data were collected from 196 patients. Steady state was achieved by cycle 1 day 15. On cycle 1 day 15, the mean (standard deviation) trough plasma concentrations was 2026 (1147) ng/mL, respectively. No consistent difference in rucaparib PK was observed between the *BRCA*-mutant, *BRCA*–wild-type/LOH-high, and *BRCA*–wild-type/LOH-low subgroups (figure S4).

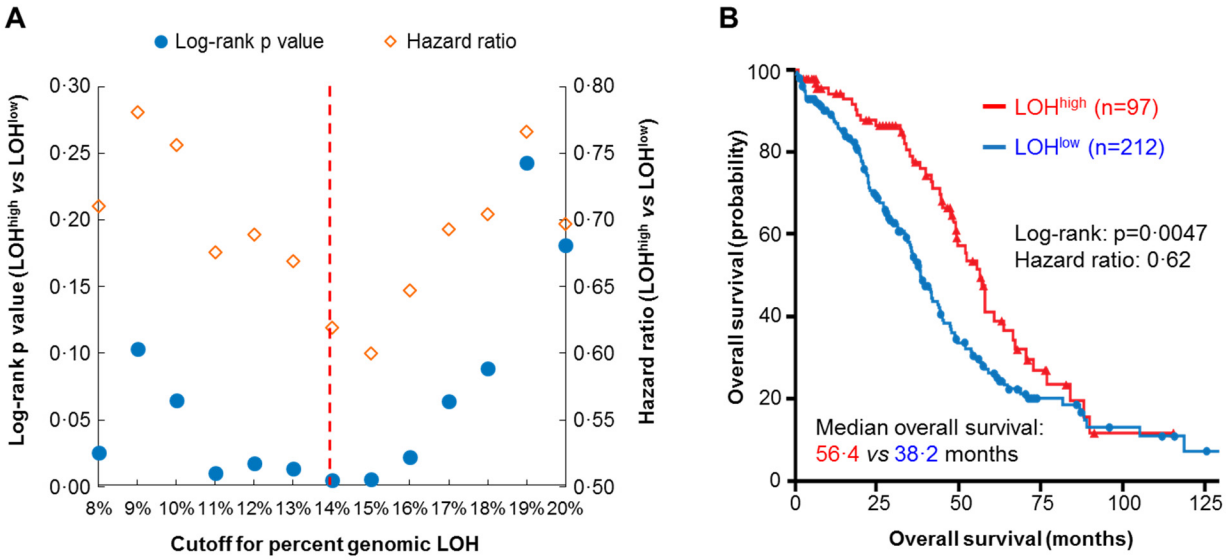
Figure S1: Study scheme



CA-125=cancer antigen 125. LOH=loss of heterozygosity. RECIST=Response Evaluation Criteria In Solid Tumors version 1·1.

Figure S2: Prespecification of percent genomic LOH cutoff based on the TCGA high-grade serous ovarian carcinoma analysis dataset

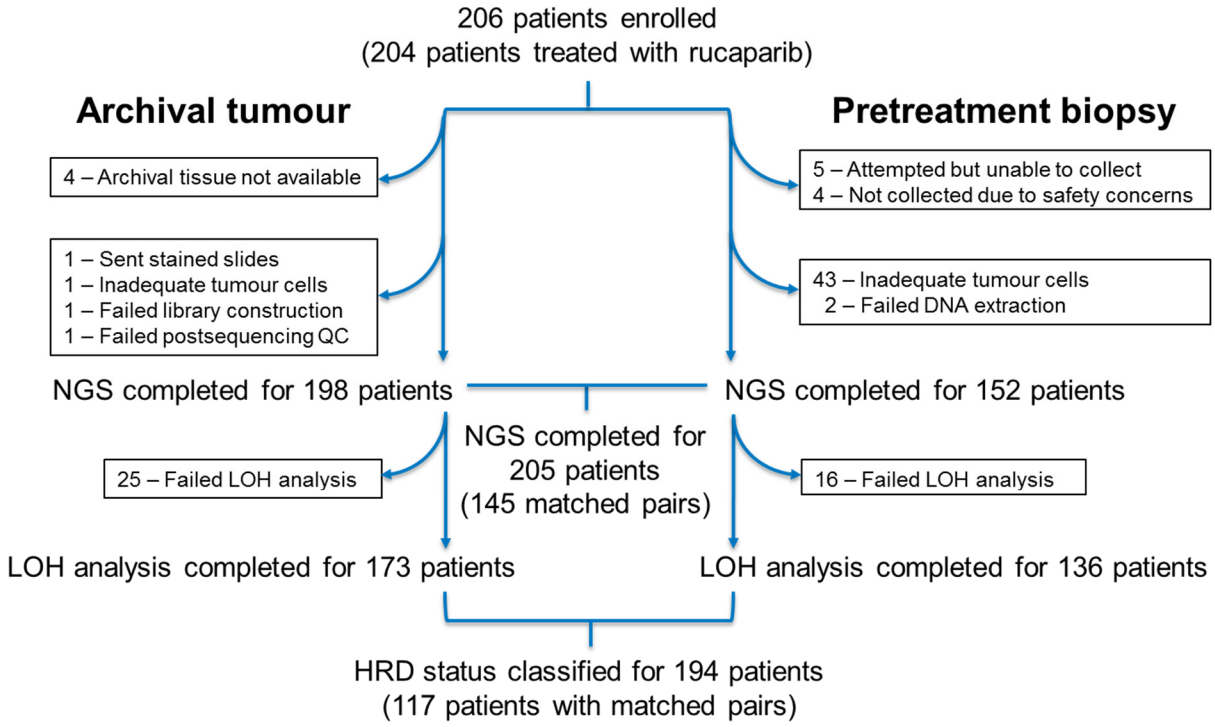
(**A**) Log-rank p values (blue filled dots, primary y-axis) and hazard ratios (orange unfilled dots, secondary y-axis) from Kaplan-Meier overall survival analysis of patients with LOH-high versus LOH-low tumours tested at different percent genomic LOH cutoffs. (**B**) Kaplan-Meier overall survival analysis of patients with LOH-high (red curve) versus LOH-low (blue curve) tumours using the 14% genomic LOH cutoff.



LOH=loss of heterozygosity. TCGA=The Cancer Genome Atlas.

Figure S3: Patient sample processing flowchart of NGS-based HRD assay in ARIEL2

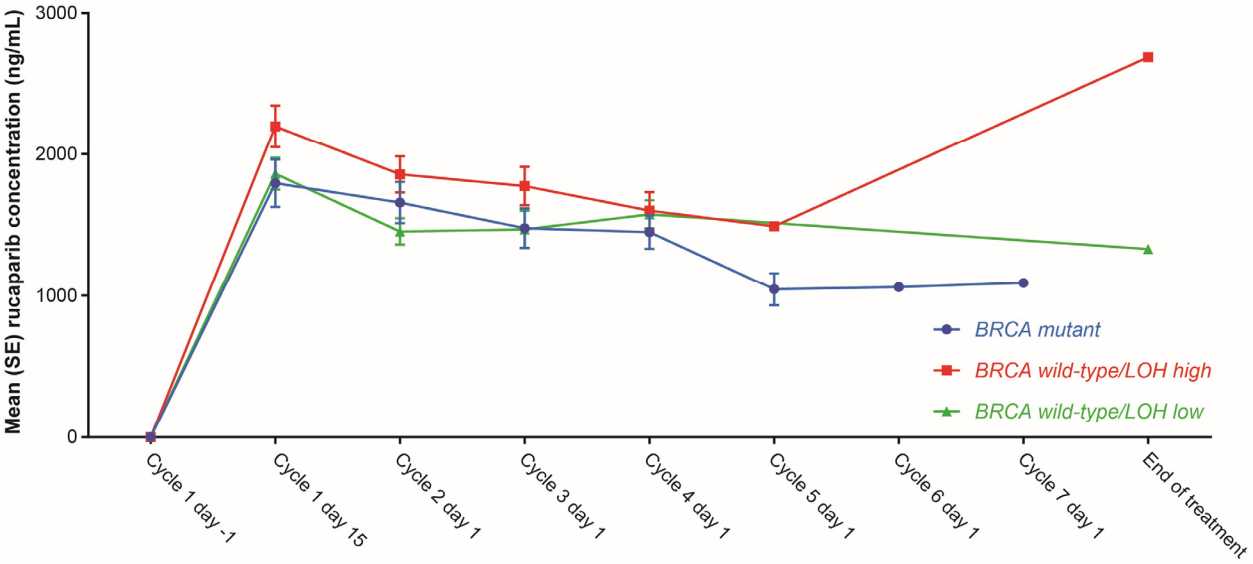
Sample attrition and QC failures are listed for the key steps of the tissue collection, NGS, and genomic LOH analysis process.



LOH=loss of heterozygosity. NGS=next-generation sequencing. QC=quality control.

Figure S4: Rucaparib trough plasma concentration versus time

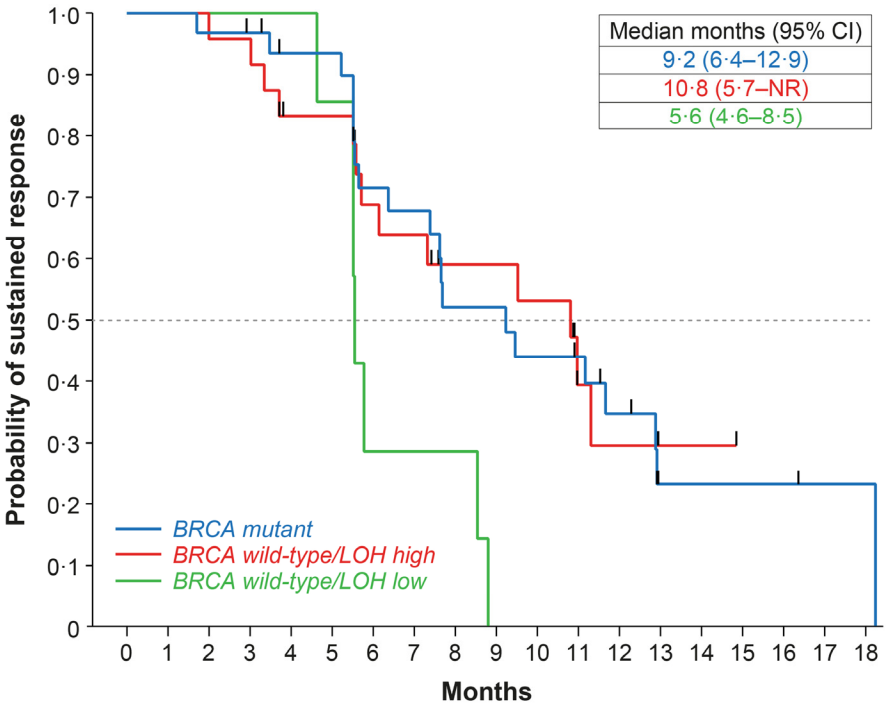
Data presented are the mean (SE) trough plasma concentrations over time in the patients who received rucaparib and had ≥1 PK sample collected.



LOH=loss of heterozygosity; SE=standard error.

Figure S5: Duration of response by HRD subgroup

Kaplan-Meier estimates of duration of confirmed response in all patients who received at least one dose of rucaparib, stratified by HRD subgroup. Median duration of confirmed response for the *BRCA*-mutant (blue), *BRCA*–wild-type/LOH-high (red), and *BRCA*–wild-type/LOH-low (green) subgroups was 9·2 (95% CI, 6·4–12·9), 10·8 (95% CI, 5·7–not reached), and 5·6 (95% CI, 4·6–8·5) months.



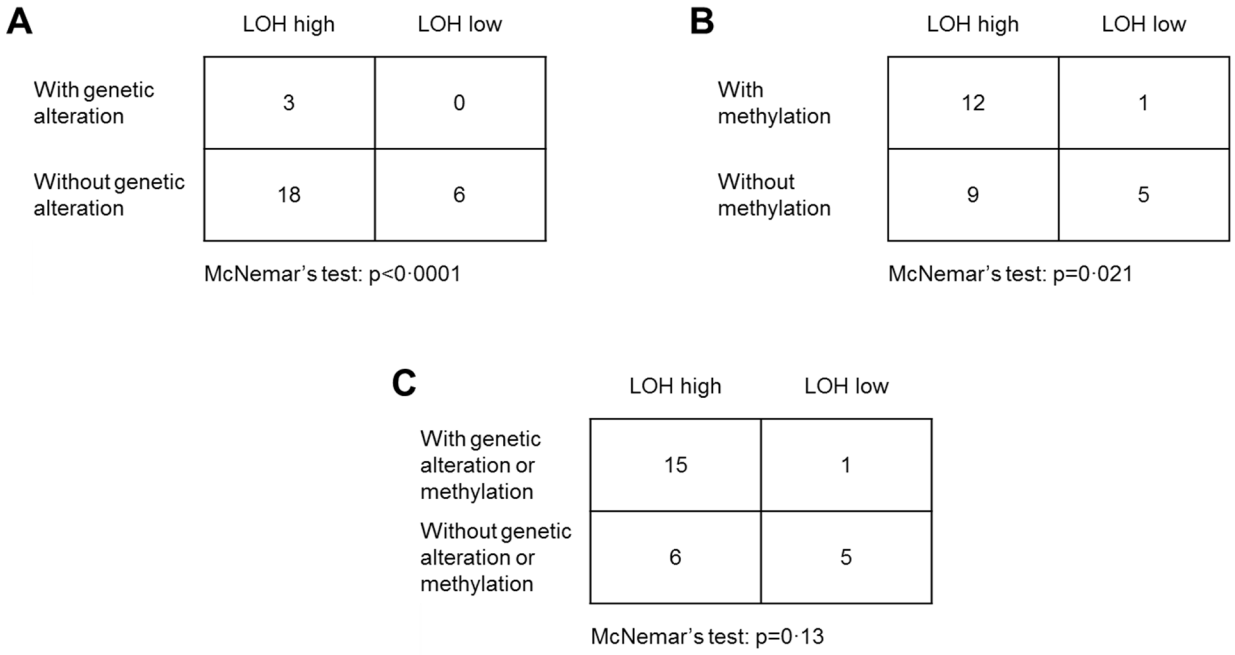
Number at risk (number censored)

BRCA mutant	32 (0)	32 (0)	31 (0)	31 (0)	28 (2)	26 (4)	25 (1)	18 (5)	18 (5)	13 (6)	13 (6)	11 (6)	10 (7)	7 (8)	6 (8)	2 (11)	2 (11)	2 (11)	1 (12)	1 (12)	0 (12)
BRCA wild-type/LOH high	24 (0)	24 (0)	24 (0)	23 (0)	21 (0)	18 (2)	18 (1)	13 (3)	12 (3)	10 (5)	10 (5)	9 (5)	4 (8)	3 (8)	3 (8)	1 (10)	1 (10)	0 (11)			
BRCA wild-type/LOH low	7 (0)	7 (0)	7 (0)	7 (0)	7 (0)	7 (0)	6 (0)	2 (0)	2 (0)	2 (0)	0 (0)										

CI=confidence interval. HRD=homologous recombination deficiency. LOH=loss of heterozygosity. NR=not reached.

Figure S6: Comparison of different biomarkers with percent genomic LOH in patients with *BRCA*–wild-type tumours with RECIST responses

Contingency tables for comparisons of three different methods of molecular biomarker classification: (**A**) homologous recombination gene mutation, (**B**) *BRCA1* or *RAD51C* methylation, and (**C**) combined mutation/methylation, with percent LOH classification. Sensitivity to detect RECIST responders with *BRCA*–wild-type tumours using the different biomarkers was 78% for genomic LOH, 11% for homologous recombination gene mutation, 48% for *BRCA1* or *RAD51C* methylation, and 59% for combined mutation/methylation. Of the 31 patients with *BRCA*–wild-type tumours with RECIST responses, complete data for all four biomarkers were available for 27 patients to enable comparisons using the McNemar's test.

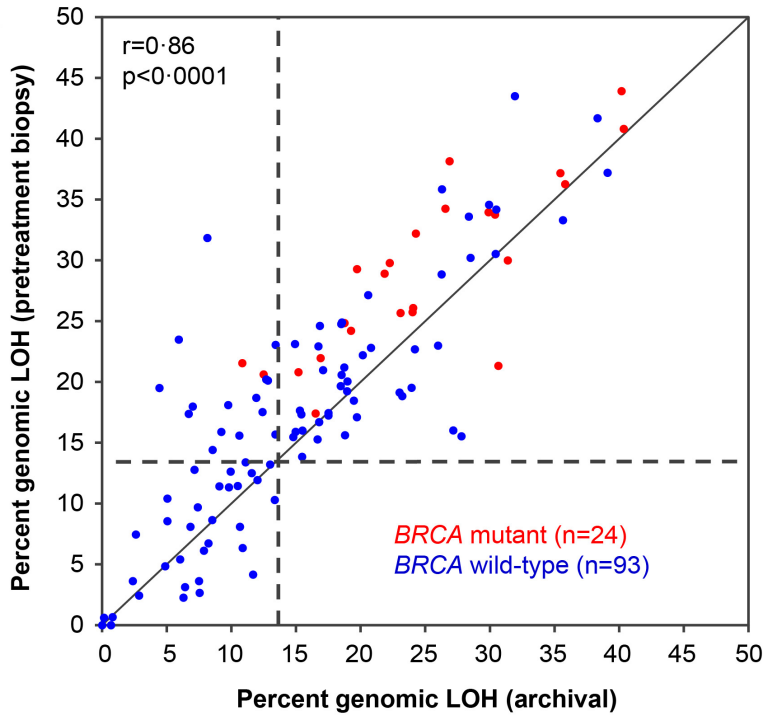


LOH=loss of heterozygosity. RECIST=Response Evaluation Criteria In Solid Tumors.

Figure S7: Assessment of genomic LOH in archival tumours and pretreatment biopsies sequenced in ARIEL2

(**A**) Percent of genomic LOH in matched archival and pretreatment biopsies. *BRCA*-mutant (red data points) and *BRCA*–wild-type tumours (blue data points) are indicated. Dashed lines indicate the prespecified genomic LOH cutoff of 14%. Solid diagonal line indicates a positive linear correlation of 1 as reference. Classification of matched archival and pretreatment biopsies (**B**) into LOH-high and LOH-low categories based on the prespecified genomic LOH cutoff of ≥14% in all patients and (**C**) into *BRCA1* methylated and unmethylated categories in patients with available methylation data.

A



B **117 matched pairs with genomic LOH data**

		Archival: LOH high	Archival: LOH low
Pretreatment biopsy	LOH high	67	17
	LOH low	0	33

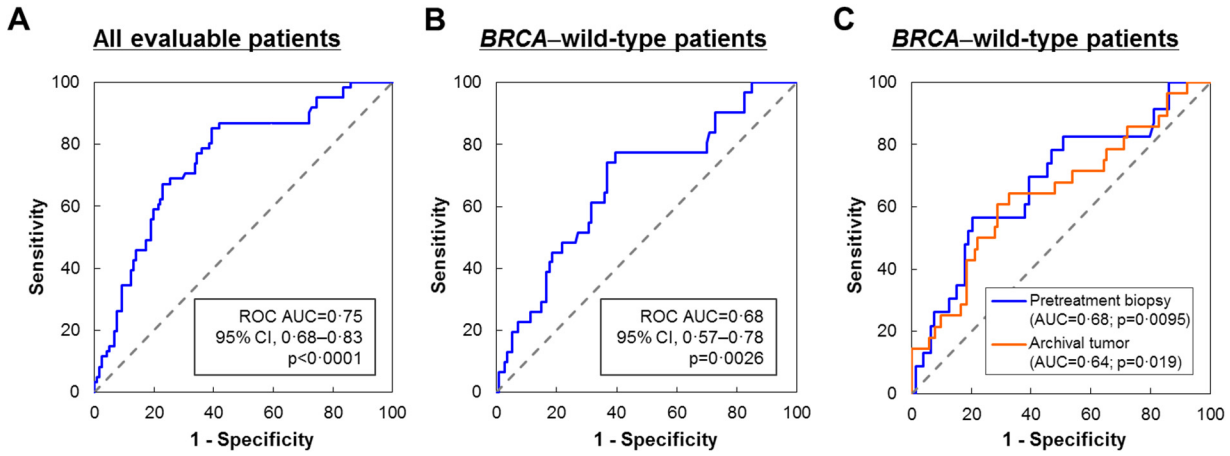
C **90 matched pairs with methylation data**

		Archival: *BRCA1* methylated	Archival: *BRCA1* unmethylated
Pretreatment biopsy	*BRCA1* methylated	9	1
	BRCA1 unmethylated	4	76

LOH=loss of heterozygosity.

Figure S8: Receiver operating characteristic analysis of the percent genomic LOH to predict RECIST responders to rucaparib

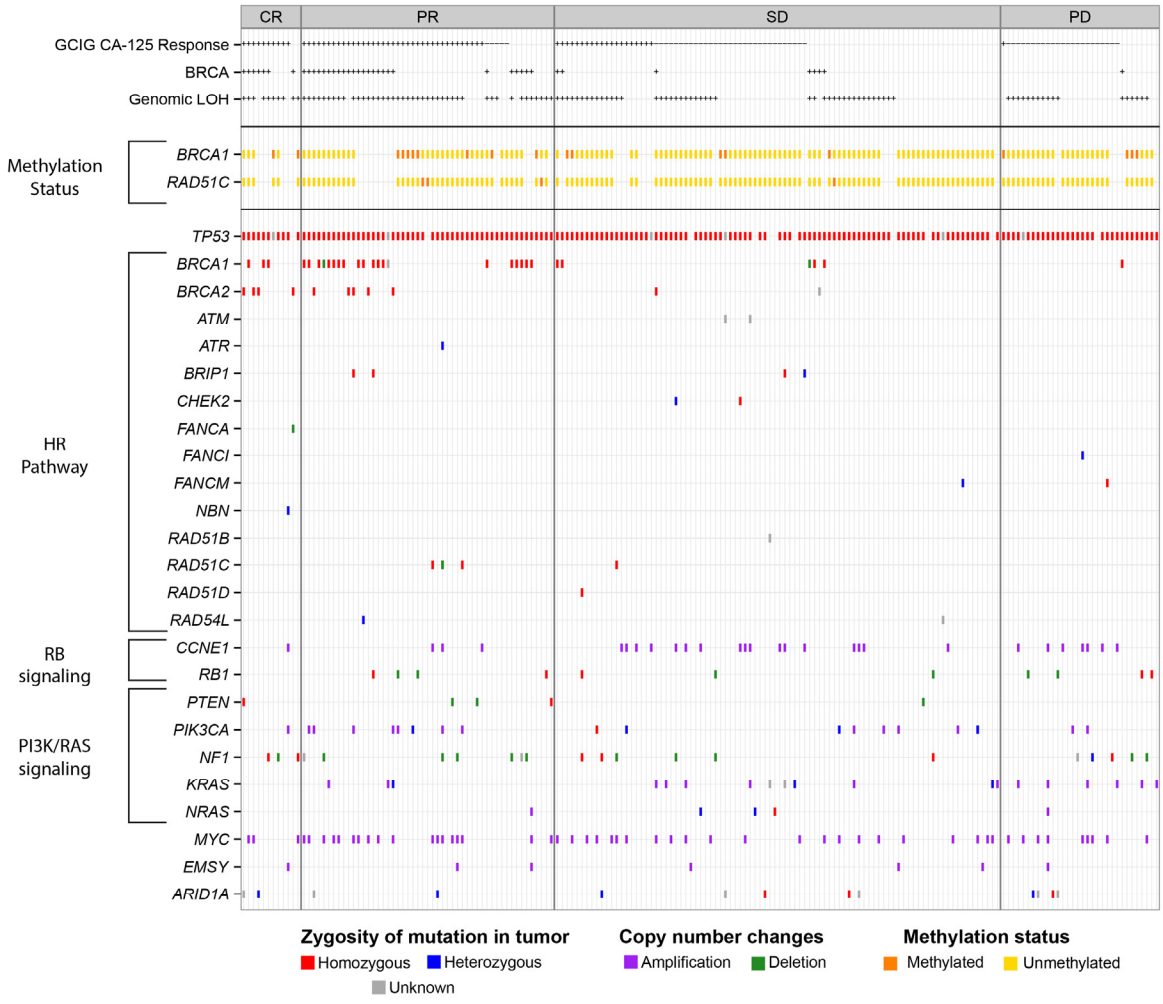
(**A**) All evaluable patients, (**B**) *BRCA*–wild-type patients, and (**C**) *BRCA*–wild-type patients using pretreatment biopsies (blue data points) versus archival tumours (orange data points).



AUC=area under the curve. CI=confidence interval. ROC=receiver operating characteristic.

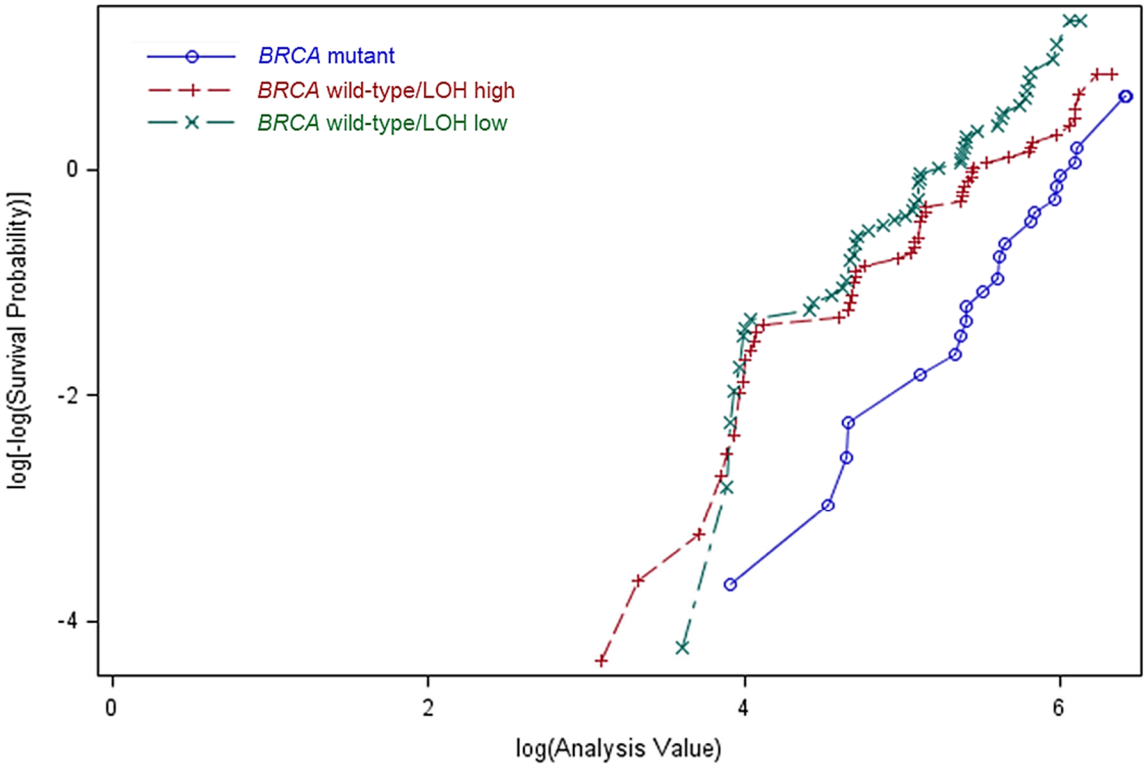
Figure S9: Genomic landscape of HRD molecular subgroups and rucaparib sensitivity

Molecular profile of the most recently collected tumour tissue specimen for each patient (each column), based on the pretreatment biopsy if available and archival tumour if biopsy not available. Patients are sorted by the following order: RECIST response, Gynecological Cancer InterGroup (GCIG) CA-125 response, and HRD subgroup. For GCIG CA-125, "+" denotes a CA-125 response, "-" denotes a lack of CA-125 response, and an empty tile denotes nonevaluable cases. For *BRCA* status, "+" indicates a *BRCA*-mutant tumour. Genetic alterations are grouped by different pathways commonly altered in high-grade ovarian carcinoma. For genomic LOH status, "+" indicates a LOH-high tumour based on the prespecified genomic LOH cutoff of ≥14%. For methylation status, an empty tile indicates no samples available to perform methylation assays. For genetic alterations, an empty tile indicates no genetic alterations detected for the specific gene. For the known and putative tumour suppressor genes in the homologous recombination pathway, only genetic alterations that result in protein truncations or homozygous deletions ("Deletion") are shown. The zygosity of each mutation identified in tumour is classified as homozygous (indicating biallelic inactivation), heterozygous, or unknown zygosity. For the genes in the other signalling pathways, only genetic alterations with known or likely functional impact based on the COSMIC database are shown.



CA-125=cancer antigen 125. CR=complete response. HR=homologous recombination. LOH=loss of heterozygosity. PR=partial response. SD=stable disease. PD=progressive disease.

Figure S10: Log of the cumulative hazard for PFS by HRD subgroup



HRD=homologous recombination deficiency. LOH=loss of heterozygosity. PFS=progression-free survival.

Table S1: Inclusion and exclusion criteria for ARIEL2

Patients were eligible for ARIEL2 if the following criteria were met:
1. Signed an Institutional Review Board/Independent Ethics Committee–approved informed consent form prior to any study-specific evaluation
2. Age ≥18 years
3. Had a histologically confirmed diagnosis of high-grade serous or grade 2 or grade 3 endometrioid epithelial ovarian, fallopian tube, or primary peritoneal cancer a. If mixed histology, >50% of the primary tumour had to be confirmed to be high-grade serous or endometrioid upon re-review by local pathology
4. Had relapsed/progressive disease as confirmed by radiologic assessment
5. Had received prior platinum-based therapy and had platinum-sensitive disease a. Received ≥1 prior platinum-based treatment regimen; AND b. Received a platinum-based regimen as their last treatment; continuous or switch maintenance treatment as part of this regimen was permitted (hormonal treatment may have been permitted following the last platinum regimen with advance approval from the sponsor); AND c. Was sensitive to the last platinum regimen. Platinum-sensitive disease was defined as documented radiologic progression ≥6 months after the last dose of platinum administered in the treatment setting
6. If <55 years of age at diagnosis, or had prior history of breast cancer, or had close relative (first or second degree) with ovarian cancer or early onset (age <50 years) breast cancer, must have been previously tested for germline *BRCA* mutation
7. Had undergone a biopsy of tumour tissue prior to first dose of study drug and had the tumour tissue confirmed by the central laboratory as being of adequate quality (≥20% tumour content with ≥80% nucleated cellular content) a. If tumour tissue obtained from the biopsy was deemed inadequate and the patient was unwilling or unable to have another biopsy, the patient may have been considered for enrolment if archival tumour tissue was provided and deemed of adequate quality prior to any treatment with rucaparib i. Biopsy had to be of solid tumour tissue; ascites was not acceptable ii. Biopsy must have been of sufficient yield for planned analyses
8. Had sufficient archival FFPE tumour tissue available for planned analyses; cytospin blocks from ascites were not acceptable a. The most recently obtained tumour tissue of adequate quality (≥20% tumour content with ≥80% nucleated cellular content) was to be submitted
9. Had measurable disease as defined by RECIST v1·1 in addition to the lesion planned for biopsy; a single RECIST target lesion was sufficient if, in the investigator's opinion, it was of sufficient size that the biopsy would not affect postdose RECIST evaluations
10. Had adequate organ function confirmed by the following laboratory values obtained within 14 days prior to the first dose of rucaparib: a. Bone marrow function i. Absolute neutrophil count ≥1·5×10^9/L ii. Platelets >100×10^9/L iii. Haemoglobin ≥9 g/dL b. Hepatic function i. Aspartate aminotransferase and alanine aminotransferase ≤3× upper limit of normal (ULN); if liver metastases, then ≤5×ULN ii. Bilirubin ≤1·5×ULN (<2×ULN if hyperbilirubaemia is due to Gilbert's syndrome) c. Renal function i. Serum creatinine ≤1·5×ULN or estimated glomerular filtration rate ≥45 mL/min using the Cockcroft-Gault formula
11. Had an Eastern Cooperative Oncology Group Performance Status of 0 to 1
Patients were excluded from ARIEL2 if any of the following criteria applied:
1. Pretreatment solid tumour biopsy AND formalin-fixed paraffin-embedded archival tissue of insufficient quality, defined as tumour nuclei of ≥20% and volume of ≥0·2 mm^3
2. Active second malignancy, ie, patient known to have potentially fatal cancer present for which she was currently (but not necessarily) receiving treatment a. Patients with a history of malignancy that has been completely treated, with no current evidence of that cancer, were permitted to enrol in the trial provided all chemotherapy was completed >6 months prior and/or bone marrow transplant was completed >2 years prior to first dose of rucaparib
3. Previous treatment with any PARP inhibitor, including oral or intravenous rucaparib; patients who previously received iniparib were eligible
4. Symptomatic and/or untreated CNS metastases; patients with asymptomatic previously treated CNS metastases were eligible provided they had been clinically stable for ≥4 weeks
5. Preexisting duodenal stent and/or any gastrointestinal disorder or defect that would, in the opinion of the investigator, interfere with absorption of rucaparib
6. Known human immunodeficiency virus or acquired immunodeficiency syndrome–related illness, or history of chronic hepatitis B or C
7. Pregnant or breast feeding; women of childbearing potential must have had a negative serum pregnancy test <3 days prior to first dose of rucaparib
8. Received treatment with chemotherapy, radiation, antibody therapy or other immunotherapy, gene therapy, vaccine therapy, angiogenesis inhibitors, or experimental drugs ≤14 days prior to first dose of rucaparib and/or had ongoing adverse effect from such treatment that was NCI CTCAE grade >1 (ongoing grade 2 nonhaematologic toxicity related to most recent treatment regimen allowed with prior advanced approval from study sponsor)
9. Received administration of strong CYP1A2 or CYP3A4 inhibitors ≤7 days prior to first dose of rucaparib or had ongoing requirements for these medications

10.	Nonstudy-related minor surgical procedure ≤5 days or major surgical procedure ≤21 days prior to first dose of rucaparib; in all cases, the patient must have sufficiently recovered and stabilised before treatment administration
11.	Presence of any other condition that could increase the risk associated with study participation or could interfere with the interpretation of study results, and, in the opinion of the investigator, would make the patient inappropriate for entry into the study
12.	Diagnosis of low-grade serous or grade 1 endometrioid ovarian cancer

CNS=central nervous system. CYP=cytochrome P450. FFPE=formalin-fixed, paraffin-embedded. NCI CTCAE=National Cancer Institute Common Terminology Criteria for Adverse Events. RECIST v1·1=Response Evaluation Criteria In Solid Tumors version 1·1.

Table S2: Multivariate analysis of percent genomic LOH as a predictor of overall survival in TCGA serous high-grade ovarian carcinoma dataset

		Hazard ratio	p value
Percent genomic LOH	LOH high	0·69	0·04
	LOH low	1·00	
BRCA mutation status	*BRCA* mutant	0·52	<0·001
	BRCA wild-type	1·00	
Tumour residual disease	No macroscopic disease	0·44	0·004
	1–10 mm	0·81	0·30
	11–20 mm	0·78	0·49
	>20 mm	1·00	
LOH=loss of heterozygosity. TCGA=The Cancer Genome Atlas.			

Table S3: Evidence supporting genes involved in homologous recombination pathway and in vitro PARP inhibitor sensitivity

Gene symbol	Gene family/functional role in homologous recombination repair	Reference(s) citing role in homologous recombination repair	Reference(s) showing in vitro sensitivity to PARP inhibitors
BRCA1	*BRCA* genes	Moynahan, Mol Cell 1999	Farmer, Nature 2005; Lord, DNA Repair 2008
BRCA2	*BRCA* genes	Xia, PNAS 2001	Bryant, Nature 2005; Farmer, Nature 2005; Lord, DNA Repair 2008
ATM	DNA damage response genes	Beucher, EMBO J 2009	McCabe, Cancer Res 2006; Turner, EMBO 2008; Weston, Blood 2010; Murai, Cancer Res 2012; Shen, CCR 2013
ATR	DNA damage response genes	Chanoux, J Biol Chem 2009	McCabe, Cancer Res 2006; Lord, DNA Repair 2008; Turner, EMBO 2008
ATRX	Helicase that regulates homologous recombination repair	Lovejoy, PLoS Genet 2012	
BARD1	BRCA1 protein complex	Westermark, Mol Cell Biol 2003	
BLM	Helicase that regulates homologous recombination repair	Ellis, Cell 1995	Gottipati, Cancer Res 2010; Murai, Cancer Res 2012
BRIP1	Helicase that regulates homologous recombination repair	Litman, Cancer Cell 2005	
CHEK1	DNA damage response genes	Sorensen, Nat Cell Biol 2005	McCabe, Cancer Res 2006; Turner, EMBO 2008; Shen, CCR 2013
CHEK2	DNA damage response genes	Zhang, Mol Cell Biol 2004	McCabe, Cancer Res 2006
FANCA	Fanconi anaemia genes	Yang, Carcinogenesis 2005	McCabe, Cancer Res 2006
FANCC	Fanconi anaemia genes	Niedzwiedz, Mol Cell 2004	McCabe, Cancer Res 2006; Murai, Cancer Res 2012
FANCD2	Fanconi anaemia genes	Ohashi, J Biol Chem 2005	McCabe, Cancer Res 2006; Murai, Cancer Res 2012
FANCE	Fanconi anaemia genes	Venkitaraman, NEJM 2003	
FANCF	Fanconi anaemia genes	Venkitaraman, NEJM 2003	
FANCG	Fanconi anaemia genes	Yamamoto, Mol Cell Biol 2003	Murai, Cancer Res 2012
FANCI	Fanconi anaemia genes	Smogorzewska, Cell 2007	
FANCL	Fanconi anaemia genes	Meetei. Cell Cycle 2004	
FANCM	Fanconi anaemia genes	Gari, Mol Cell 2008	Shen, CCR 2013
MRE11A	MRN DSB repair complex	Bressan, Mol Cell Biol 1999	Vilar, Cancer Res 2011
NBN	MRN DSB repair complex	Tauchi, Nature 2002	McCabe, Cancer Res 2006
PALB2	Fanconi anaemia genes	Buisson, Nat Struct Mol Biol 2010	Buisson, Nat Struct Mol Biol 2010; Shen, CCR 2013
RAD50	*RAD* genes	Bressan, Mol Cell Biol 1999	
RAD51	*RAD* genes	Shinohara, Cell 1992	McCabe, Cancer Res 2006; Lord, DNA Repair 2008; Shen, CCR 2013
RAD51B	*RAD* genes	Takata, Mol Cell Biol 2000	
RAD51C	*RAD* genes	Kurumizaka, PNAS 2001	Min, Mol Cancer Ther 2013
RAD51D	*RAD* genes	Kurumizaka, J Biol Chem 2002	Loveday, Nat Genet 2011
RAD52	*RAD* genes	Lisby, PNAS 2001	Gottipati, Cancer Res 2010
RAD54L	*RAD* genes	Sigurdsson, J Biol Chem 2003	Gottipati, Cancer Res 2010; McCabe, Cancer Res 2006
RPA1	Single-stranded DNA binding complex	Wang, Nat Genetics 2005	McCabe, Cancer Res 2006

DSB=double-strand break.

Table S4: Detected mutations in non-*BRCA* homologous recombination genes and RECIST response

Gene	Mutation effect	Mutation type	Germline/ somatic	Genomic LOH status	RECIST response	CA-125 response
ATM	p.G2644fs*2	Frameshift	Somatic	LOH high	NE	NE
ATM	Homozygous deletion	CNA	Somatic	Indeterminate	SD	Yes
BRIP1	p.K752fs*12	Frameshift	Germline	LOH low	SD	No
BRIP1	Splice site c.93+1G>T	Splice	Germline	LOH low	SD	No
CHEK2	Splice site c.1008_1008+1GG>TT	Splice	Indeterminate	LOH low	SD	No
CHEK2	p.Q83fs*27	Frameshift	Germline	LOH high	SD	No
FANCA	Homozygous deletion	CNA	Somatic	LOH high	SD	NE
FANCI	p.I466fs*7	Frameshift	Germline	LOH low	PD	No
FANCM	p.Q1701*	Nonsense	Germline	LOH low	PD	No
FANCM	p.R1931*	Nonsense	Germline	LOH low	SD	NE
NBN	p.K219fs*16	Frameshift	Germline	LOH low	CR	Yes
NBN	p.K233fs*5	Frameshift	Germline	Indeterminate	SD	NE
RAD51B	p.R47*	Nonsense	Germline	LOH low	SD	No
RAD51C	p.R193*	Nonsense	Germline	LOH high	PR	Yes
RAD51C	Homozygous deletion[†]	CNA	Germline	LOH high	PR	Yes
RAD51C	Splice site c.572-2A>G	Splice	Germline	LOH high	PR	Yes
RAD51C	Splice site c.837+1G>T	Splice	Germline	LOH high	SD	Yes
RAD51D	p.G146fs*50	Frameshift	Indeterminate	LOH high	SD	Yes
RAD51D	p.R141*	Nonsense	Germline	LOH high	SD	No
RAD54L	p.F591fs*1	Frameshift	Somatic (subclonal)	LOH low	SD	NE

CA-125=cancer antigen 125. CNA=copy number alteration. CR=complete response. LOH=loss of heterozygosity. NE=not evaluable. PD=progressive disease. PR=partial response. RECIST=Response Evaluation Criteria In Solid Tumors version 1·1. SD=stable disease.
[†]A *RAD51C* p.R370* mutation near the C-terminus was detected in a minor subclone, which is likely to be insignificant compared with the primary germline homozygous deletion event in 5 of 9 exons.

Table S5: Serious adverse events

Serious adverse event	n* (%)
Any serious adverse event	50 (24·5)
Malignant neoplasm progression	10 (4·9)
Small intestinal obstruction	10 (4·9)
Anaemia	9 (4·4)
Acute kidney injury	6 (2·9)
Sepsis	4 (2·0)
Urinary tract infection	4 (2·0)
Nausea	3 (1·5)
Ascites	2 (1·0)
Neutropenia/decreased neutrophil count	2 (1·0)
Pleural effusion	2 (1·0)
Pneumonia	2 (1·0)
Vomiting	2 (1·0)
Abdominal pain	1 (0·5)
ALT/AST increased	1 (0·5)
Asthenia	1 (0·5)
Bile duct obstruction	1 (0·5)
Blood bilirubin increased	1 (0·5)
Blood cholesterol increased	1 (0·5)
Blood creatinine increased	1 (0·5)
Bronchitis	1 (0·5)
Constipation	1 (0·5)
Dehydration	1 (0·5)
Diarrhoea	1 (0·5)
Dyspnoea	1 (0·5)
Empyema	1 (0·5)
Fall	1 (0·5)
Febrile neutropenia	1 (0·5)
Humerus fracture	1 (0·5)
Hypercholesterolaemia	1 (0·5)
Ileus	1 (0·5)
Intestinal obstruction	1 (0·5)
Intestinal perforation	1 (0·5)
Large intestinal obstruction	1 (0·5)
Long QT syndrome congenital	1 (0·5)
Lower gastrointestinal haemorrhage	1 (0·5)
Lower respiratory tract infection	1 (0·5)
Lymphangitis	1 (0·5)
Lymphocyte count decreased	1 (0·5)
Mental status changes	1 (0·5)
Neutropenia	1 (0·5)
Peritonitis	1 (0·5)
Pulmonary embolism	1 (0·5)
Pyrexia	1 (0·5)
Syncope	1 (0·5)
Transaminases increased	1 (0·5)
Weigh decreased	1 (0·5)

ALT=alanine transaminase. AST=aspartate transaminase.
*For the safety population (n=204).

Table S6: Treatment-emergent adverse events leading to dose reduction in ≥1% patients

Adverse event	n* (%)
Any adverse event leading to dose reduction	80 (39·2)
Anaemia	28 (13·7)
Nausea	22 (10·8)
Asthenia/fatigue	18 (8·8)
ALT/AST increased	14 (6·9)
Vomiting	11 (5·4)
Thrombocytopenia/decreased platelets	7 (3·4)
Decreased appetite	6 (2·9)
Dysgeusia	6 (2·9)
Blood creatinine increased	5 (2·5)
Neutropenia/decreased neutrophil count	5 (2·5)
Decreased haemoglobin	4 (2·0)
Dizziness	3 (1·5)
Acute kidney injury	2 (1·0)
Diarrhoea	2 (1·0)
Gamma-glutamyltransferase increased	2 (1·0)
Headache	2 (1·0)
Hypophosphataemia	2 (1·0)

ALT=alanine transaminase. AST=aspartate transaminase.
*For the safety population (n=204).

Table S7: Treatment-emergent adverse events leading to discontinuation

Adverse event	Number of patients*
Any adverse event leading to discontinuation	19
Asthenia/fatigue	6
Abdominal pain	2
Anaemia	2
Sepsis	2
Malignant neoplasm progression	3
Back pain	1
Blood creatinine increased	1
Decreased appetite	1
Diarrhoea	1
Dizziness	1
Dysgeusia	1
Dyspnoea	1
Eyelid oedema	1
Febrile neutropenia	1
Haematochezia	1
Hydronephrosis	1
Nausea	1
Rectal haemorrhage	1
Small intestinal obstruction	1
Vomiting	1
*For the safety population (n=204); a patient may have discontinued as a result of more than one adverse event.	

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A Phase 2, Open-Label Study of Rucaparib in Patients with Platinum-Sensitive, Relapsed, High-Grade Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer

Protocol Number:	CO-338-017
Investigational Product:	Oral rucaparib (CO-338)
Eudra CT Number:	2013-000517-20
IND Number:	106,289
Development Phase:	Phase 2
Indication Studied:	Platinum-sensitive, relapsed, high-grade epithelial ovarian, fallopian tube, or primary peritoneal cancer
Sponsor Name and Address:	Clovis Oncology, Inc. 2525 28th Street, Suite 100 Boulder, CO 80301 U.S.A. Phone Number: 303-625-5000 Facsimile Number: 303-245-0360
Responsible Medical Officer:	Andrew Allen, M.D., Ph.D.
Compliance Statement:	This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, clinical research guidelines established by the Code of Federal Regulations (Title 21, CFR Parts 50, 56, and 312), and International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guidelines. Essential study documents will be archived in accordance with applicable regulations.
Protocol Date:	May 7, 2013
Amendment 1 Date:	August 19, 2013
Amendment 2 Date:	May 9, 2014
Amendment 2.1 Date:	May 30, 2014
Amendment 3 Date:	December 17, 2014
Amendment 4 Date:	December 19, 2014

Protocol Approval Signature Page

Protocol: CO-338-017

Title: A Phase 2, Open-Label Study of Rucaparib in Patients with Platinum-Sensitive, Relapsed, High-Grade Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer

Date: December 19, 2014

Reviewed and Approved by:

Andrew Allen, M.D., Ph.D. Chief Medical Officer, Executive Vice President of Clinical and Preclinical Development Clovis Oncology, Inc	Date
Gillian Ivers-Read Chief Regulatory Officer, Executive Vice President of Technical Operations Clovis Oncology, Inc.	Date
Jeff Isaacson, Ph.D. Senior Director, Biostatistics and Data Management Clovis Oncology, Inc.	Date

Protocol Acceptance Form

Protocol:	CO-338-017
Title:	A Phase 2, Open-Label Study of Rucaparib in Patients with Platinum-Sensitive, Relapsed, High-Grade Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer
Date:	December 19, 2014

I have carefully read this protocol and agree that it contains all of the necessary information required to conduct this study. I agree to conduct this study as described and according to the Declaration of Helsinki, ICH Guidelines for GCP, and all applicable regulatory requirements.

Investigator's Signature	Date
Name (printed)	

Table of Contents

Description **Page**

List of Tables

Description **Page**

List of Figures

Description **Page**

1 SYNOPSIS

Protocol Number	CO-338-017
Title	A Phase 2, Open-Label Study of Rucaparib in Patients with Platinum-Sensitive, Relapsed, High-Grade Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Cancer
Study Phase	Phase 2
Introduction	Rucaparib is an orally available, small molecule inhibitor of poly (adenosine diphosphate [ADP]–ribose) polymerase (PARP) being developed for treatment of ovarian cancer associated with homologous recombination DNA repair deficiency. The safety and efficacy of rucaparib has been evaluated in several Phase 1 and Phase 2 studies. Normal cells repair single-strand breaks (SSBs) in DNA through base excision repair (BER). While there are several variations of BER, all pathways rely on PARP enzymes, of which PARP-1 is the best characterized. SSBs that are not repaired result in stalled replication forks and the development of double-strand breaks (DSBs), which are repaired by homologous recombination repair (HRR) of DNA, a complex process involving multiple proteins, including those encoded by breast cancer susceptibility gene 1 and 2 (*BRCA1* and *BRCA2*), as well as RAD51, Fanconi anemia core complex, ataxia telangiectasia mutated (ATM), and ataxia telangiectasia and RAD3-related (ATR) protein, among others. HRR pathway defects, either as an initiating event or late event in the carcinogenetic process, may be responsible for the genetic instability observed in many cancers. The Cancer Genome Atlas (TCGA), which completed an analysis of molecular changes associated with high-grade serous ovarian cancer (HGSOC), estimated that approximately 50% of patients with HGSOC have homologous recombination deficiency (HRD).[1] Germline mutations in the *BRCA1* and *BRCA2* genes (*gBRCA*) are the strongest known hereditary factors for epithelial ovarian cancer (EOC), accounting for up to 15% of all EOC.[2,3] These patients carry heterozygous deleterious mutations in their germline DNA, and develop tumors when the remaining wild-type functional allele is inactivated (i.e., "second hit"). Approximately 6 – 8% of HGSOC patients have somatic mutations in *BRCA1* or *BRCA2* (*sBRCA*).[1,4] HRD is not limited to mutations of *BRCA1*/2, however. Approximately 27% of HGSOC patients are estimated to have HRD due either to an alteration in a HRR gene other than *BRCA1/2* or due to other molecular alteration or modification (e.g., epigenetic silencing). Inhibition of DNA damage repair in cancer cells, which are intrinsically genetically unstable, represents an attractive opportunity for the development of new therapies. Given the overlap in various DNA repair pathways, inhibition of a single pathway is unlikely to have a significant effect. Inhibition of multiple pathways, such as BER with a PARP inhibitor, in the context of tumor with intrinsic HRD, may lead to cell death, a concept known as synthetic lethality. Normal cells, with only one DNA repair pathway affected by inhibition of PARP, still have an intact DNA repair pathway that can compensate. This concept of synthetic lethality has been demonstrated in key *in vitro* and *in vivo*

Introduction (cont)	studies, as well as in several clinical trials with PARP inhibitors.[5-10] While up to 15% of patients may have a hereditary form of ovarian cancer (based on germline mutations), the majority of cases are sporadic (based on somatic mutations).[1,11-13] Both *gBRCA* and *sBRCA* mutations result in HRD, and patients whose tumors harbor these mutations derive clinical benefit from PARP inhibitor therapy.[14] Collectively, these mutations comprise a group known as tissue BRCA (tBRCA). Patients without evidence of a *gBRCA* or *sBRCA* mutation also derive benefit from PARP inhibitor treatment.[14,15] The molecular signature associated with PARP inhibitor response in a non-BRCA setting is not yet fully understood, but may be linked to other mechanisms of HRD, termed non-BRCA HRD (nbHRD). This molecular signature, as well as s*BRCA* mutations, cannot be characterized by a blood-based diagnostic test. The purpose of this study is to define a tumor-based molecular signature of HRD in ovarian cancer that correlates with response to rucaparib and enables selection of appropriate ovarian cancer patients for treatment with rucaparib. Through a series of experiments and data analyses, the Sponsor has determined that measuring the extent of genomic scarring, a downstream consequence of HRD, is a potential method for identifying patients who may be sensitive to rucaparib. Genomic scarring can be assessed by quantifying the extent of loss of heterozygosity across the tumor genome (tumor genomic LOH). One of the main advantages of detecting tumor genomic LOH is that it can identify HRD tumors regardless of the underlying mechanisms, which include both known (i.e., *BRCA* mutations) and unknown genetic and other mechanisms. In this study, patients will be prospectively placed into 1 of 3 HRD subgroups prior to primary efficacy analysis. HRD subgroups include: tBRCA (HRD related to a deleterious *BRCA1* or *BRCA2* gene mutation in tumor tissue), nbHRD (no *BRCA1* or *BRCA2* mutation; LOH^+ - meets or exceeds a pre-specified tumor genomic LOH cutoff) or biomarker negative (no *BRCA1* or *BRCA2* mutation; LOH^- - tumor genomic LOH below the prespecifed cutoff). Additional sensitivity analyses will be performed to determine the optimal tumor genomic LOH cutoff to determine rucaparib sensitivity. Once the optimal response signature is defined, it will be prospectively applied in the final analysis of the planned Phase 3 pivotal study (CO-338-014), which will evaluate rucaparib as switch maintenance treatment following a response to platinum-based chemotherapy in a similar patient population. This Phase 2 study will also compare archival versus recently collected tumor tissue in order to validate the use of archival tumor tissue for assessment of HRD status in the planned Phase 3 study.
Study Overview	This is a two-part study that will enroll patients with relapsed, platinum-sensitive, high-grade epithelial ovarian, fallopian tube, or primary peritoneal cancer who have disease that can be biopsied and is measurable. Part 1 will enroll patients who received ≥1 prior platinum-based regimen and have platinum-sensitive disease. Part 2 will enroll patients who have received at least 3, but no more than 4, prior chemotherapy regimens. In Part 1, patients <55 years of age at diagnosis, or with prior history of breast cancer, or who have a close relative (first or second degree) with ovarian cancer or early onset (<age 50) breast cancer are required to have been previously tested for *gBRCA* mutation. Enrollment of patients known a priori to harbor a *gBRCA* mutation

Study Overview (cont)	classified as deleterious (pathogenic), suspected deleterious, or favor deleterious (or the equivalent interpretation of any of these) on the most recent assessment by a testing laboratory will be limited to 15 in Part 1 in order to enrich for patients that have HRD associated with a defect other than germline *BRCA1/2*. Patients with a *tBRCA* mutation but no *gBRCA* mutation do not count toward this cap and will be eligible to receive treatment with rucaparib, provided all other eligibility criteria are met. In Part 2, at least 80 patients with a *tBRCA* mutation will be enrolled. All patients, with the exception of Part 2 patients known to harbor a deleterious *gBRCA* mutation, will be required to undergo a pre-dose biopsy for collection of tumor tissue. Archival tumor tissue will also be collected. *tBRCA* mutation and/or tumor genomic LOH analysis will be performed using Foundation Medicine's next generation sequencing (NGS) test. Analysis of tumor genomic LOH is expected to identify tumors with HRD regardless of the underlying mechanism(s). *tBRCA* mutation and/or the extent of tumor genomic LOH will be correlated with the clinical outcome with rucaparib. The NGS test also targets a large panel of other cancer-related genes and other HRR pathway genes. Results of the Foundation Medicine panel test will be provided to all patients who consent to receive this information. In the event a *BRCA1* or *BRCA2* mutation is identified in tumor tissue, the patient may be referred by the investigator for genetic counseling and potential germline testing per institutional guidelines. If the patient chooses to have germline testing, this result will be entered in the clinical trial database for this study. Alterations detected in tumor tissue may be somatic or germline; however, the NGS test will not distinguish between the two. A blood sample will therefore be collected for all patients at screening and stored. Prior to final efficacy analysis, genomic DNA may be subjected to exploratory analysis in order to determine whether the mutation is germline or somatic. These data will be generated in a research setting and will not be provided to the investigator or patient. The following correlative translational studies are planned: 1. Tumor genomic LOH and gene sequence alterations in archival and screening tumor tissue will be compared to assess the changes in a tumor's genomic LOH and genetic profile over time and determine if archival tumor tissue carries sufficient predictive utility and obviates the need for a contemporaneous biopsy. The frequency and nature of secondary *BRCA* mutations will also be assessed. Acquired secondary *BRCA* mutations (also known as reversions) may result in functional protein and restored HRR capability, leading to PARP inhibitor resistance.[16-18] 2. An alternative NGS test known as BROCA will be used to potentially identify mutations in other DNA repair genes that may confer sensitivity or resistance to rucaparib.[19] 3. Gene expression profiling on extracted RNA will be analyzed to potentially identify a signature associated with efficacy. A gene expression signature has been developed to identify BRCA and BRCA-like (also referred to as "BRCAness") tumors. Such a signature may predict response to platinum and PARP inhibitors.[20]

Study Overview (cont)	4. Immunohistochemistry (IHC) of non-homologous end joining (NHEJ) proteins will be investigated to assess whether NHEJ pathway integrity modulates efficacy. It has been hypothesized that cells with HRD must have functional NHEJ DNA repair in order to generate sufficient genomic instability for synthetic lethality with a PARP inhibitor.[21] 5. Circulating cell-free tumor DNA (ctDNA) will be analyzed as a potential molecular marker of efficacy. Tagged-amplicon deep sequencing (TAm-Seq) will be utilized to sequence ctDNA and identify mutations, including but not limited to, those in the tumor suppressor gene TP53, which is present in greater than 95% of high-grade serous ovarian tumors.[1,22] Similar to CA-125, the fraction of TP53 mutant alleles in plasma of ovarian cancer patients has been shown to track with the clinical course of the disease.[22]
Number of Patients	**Part 1:** Approximately 180 patients will be enrolled. Patients known a priori to harbor a *gBRCA* mutation classified as deleterious (pathogenic), suspected deleterious, or favor deleterious (or the equivalent interpretation of any of these) on the most recent assessment by a testing laboratory will be limited to 15. Patients who do not harbor a known *gBRCA* mutation but are found to have a *tBRCA* mutation after their tumor tissue is analyzed by the Foundation Medicine NGS test are not subject to this cap and will be eligible to receive treatment with rucaparib. **Part 2:** Up to 300 patients will be enrolled, including at least 80 patients with a *tBRCA* mutation, as identified by the Foundation Medicine NGS test. Patients will enroll into either Part 1 or Part 2 of the study. Part 2 will begin once enrollment of Part 1 has been completed.
Number of Sites	This is a multicenter, multinational study. Patients will be enrolled from approximately 60 study sites.
Study Duration	Q4 2013 – Q4 2016 (estimated)
Study Objectives	Unless otherwise specified, the objectives apply to both parts of the study. The primary objectives of this study are: • To determine progression-free survival (PFS) in patients with relapsed platinum-sensitive ovarian cancer classified into molecularly-defined subgroups by a prospectively defined HRD signature (Part 1) • To estimate objective response rate (ORR) in heavily pre-treated patients with relapsed ovarian cancer classified into molecularly-defined subgroups by a prospectively defined HRD signature (Part 2) The secondary objectives of this study are: • To estimate ORR (Part 1) • To estimate ORR including cancer antigen 125 (CA-125) response criteria • To evaluate duration of response (DOR) • To determine PFS (Part 2) • To evaluate survival (Part 2) • To evaluate the safety and tolerability of rucaparib

Study Objectives (cont)	• To evaluate steady state trough level pharmacokinetics (PK) The exploratory objectives of this study are: • To assess efficacy in molecularly-defined HRD subgroups as defined by HRR gene alterations • To optimize the tumor LOH algorithm by testing additional signatures of interest based on higher or lower genomic LOH • To assess changes in HRD status over time • To assess whether the BROCA panel can identify mutations in additional HRR genes that may be associated with efficacy • To assess if a gene expression signature for HRD correlates with efficacy • To assess NHEJ pathway integrity and correlate it with efficacy • To assess ctDNA as a molecular marker of efficacy
Study Population	Unless otherwise specified, the criteria below apply to both parts of the study. **Inclusion Criteria** Eligible patients must meet the following inclusion criteria: 1. Have signed an Institutional Review Board/Independent Ethics Committee-approved informed consent form prior to any study-specific evaluation 2. Be ≥18 years of age at the time the informed consent form is signed 3. Have a histologically confirmed diagnosis of high-grade serous or Grade 2 or Grade 3 endometrioid epithelial ovarian, fallopian tube, or primary peritoneal cancer – If mixed histology, >50% of the primary tumor must be confirmed to be high-grade serous or endometrioid upon re-review by local pathology – Patients with a histology of other than serous or endometrioid are also eligible for Part 2 of the study if they are known to harbor a deleterious/ pathogenic *BRCA* mutation (germline or somatic) 4. Have relapsed/progressive disease as confirmed by radiologic assessment 5. **Part 1**: Received prior platinum-based therapy and have platinum-sensitive disease a. Received ≥1 prior platinum-based treatment regimen; AND b. Received a platinum-based regimen as their last treatment; continuous or switch maintenance treatment as part of this regimen is permitted (hormonal treatment may be permitted following the last platinum regimen with advance approval from the Sponsor); AND c. Was sensitive to the last platinum regimen. Platinum-sensitive disease is defined as documented radiologic progression ≥6 months after the last dose of platinum administered in the treatment setting. **Part 2**: Received at least 3, but no more than 4, prior chemotherapy regimens and had documented treatment-free interval of ≥6 months following 1st chemotherapy regimen received a. Hormonal agents (eg. tamoxifen, letrozole, etc), anti-angiogenic agents (eg. bevacizumab, pazopanib, cediranib, nintedanib, trebananib, etc),

Study Population (cont)	and other non-chemotherapy agents administered as single agent treatment will not be counted as a chemotherapy regimen for the purpose of determining patient eligibility b. Agents administered in the maintenance setting will not be counted as a separate regimen 6. **Part 1 only:** If <55 years of age at diagnosis, or has prior history of breast cancer, or has close relative (first or second degree) with ovarian cancer or early onset (<age 50) breast cancer, must have been previously tested for *gBRCA* mutation; after 15 patients harboring the *gBRCA* mutation are enrolled, no additional patients with a known *gBRCA* mutation will be allowed to enroll. 7. Have undergone a biopsy of tumor tissue prior to first dose of study drug and had the tumor tissue confirmed by the central laboratory as being of adequate quality (at least 20% tumor content with a minimum of 80% nucleated cellular content). *Note: biopsy is optional for Part 2 patients known to harbor a deleterious gBRCA mutation* – If tumor tissue obtained from the biopsy is deemed inadequate, and the patient is unwilling or unable to have another biopsy, the patient may be considered for enrollment if archival tumor tissue is provided and deemed of adequate quality. This must occur prior to any treatment with rucaparib. a. Biopsy must be of solid tumor tissue; ascites is not acceptable b. Biopsy must be of sufficient yield for planned analyses 8. Have sufficient archival formalin-fixed paraffin-embedded (FFPE) tumor tissue available for planned analyses; cytospin blocks from ascites are not acceptable – The most recently obtained tumor tissue that is of adequate quality (at least 20% tumor content with a minimum of 80% nucleated cellular content) should be submitted 9. Have measurable disease as defined by RECIST v1.1 (Appendix B) in addition to the lesion planned for biopsy; a single RECIST target lesion will suffice if, in the Investigator's opinion, it is of sufficient size that the biopsy will not affect post-dose RECIST evaluations. 10. Have adequate organ function confirmed by the following laboratory values obtained within 14 days prior to the first dose of rucaparib: a. Bone Marrow Function i. Absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/L ii. Platelets $>100 \times 10^9$/L iii. Hemoglobin ≥9 g/dL b. Hepatic Function i. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤3 × upper limit of normal (ULN); if liver metastases, then ≤5 × ULN

Study Population (cont)	ii. Bilirubin ≤1.5 × ULN; <2 × ULN if hyperbilirubemia is due to Gilbert's syndrome iii. Serum albumin ≥30 g/L (3.0 g/dL) (Part 2 only) c. Renal Function i. Serum creatinine ≤1.5 x ULN or estimated glomerular filtration rate (GFR) ≥45 mL/min using the Cockcroft Gault formula 11. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1 (Appendix C) **Exclusion Criteria** Patients will be excluded from participation if any of the following criteria apply: 1. Active second malignancy, i.e., patient known to have potentially fatal cancer present for which she may be (but not necessarily) currently receiving treatment a. Patients with a history of malignancy that has been completely treated, with no evidence of that cancer currently, are permitted to enroll in the trial provided all chemotherapy was completed >6 months prior and/or bone marrow transplant (BMT) >2 years prior to first dose of rucaparib 2. Prior treatment with any PARP inhibitor, including oral or intravenous rucaparib. Patients who previously received iniparib are eligible. 3. Symptomatic and/or untreated central nervous system (CNS) metastases. Patients with asymptomatic previously treated CNS metastases are eligible provided they have been clinically stable for at least 4 weeks. 4. Pre-existing duodenal stent and/or any gastrointestinal disorder or defect that would, in the opinion of the Investigator, interfere with absorption of rucaparib 5. Known human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS)-related illness, or history of chronic hepatitis B or C 6. Pregnant or breast feeding. Women of childbearing potential must have a negative serum pregnancy test <3 days prior to first dose of rucaparib. 7. Received treatment with chemotherapy, radiation, antibody therapy or other immunotherapy, gene therapy, vaccine therapy, angiogenesis inhibitors, or experimental drugs ≤14 days prior to first dose of rucaparib and/or ongoing adverse effects from such treatment > NCI CTCAE Grade 1 (ongoing Grade 2 non-hematologic toxicity related to most recent treatment regimen may be permitted with prior advanced approval from Sponsor) 8. Received administration of strong CYP1A2 or CYP3A4 inhibitors ≤7 days prior to first dose of rucaparib or have on-going requirements for these medications (Appendix D) 9. Non-study related minor surgical procedure ≤5 days, or major surgical procedure ≤21 days, prior to first dose of rucaparib; in all cases, the patient must be sufficiently recovered and stable before treatment administration 10. Presence of any other condition that may increase the risk associated with study participation or may interfere with the interpretation of study results,

Study Population (cont)	and, in the opinion of the investigator, would make the patient inappropriate for entry into the study 11. Diagnosis of low-grade serous or Grade 1 endometrioid ovarian cancer **Part 2 Only** 12. Hospitalization for bowel obstruction within 3 months prior to enrollment Pregnancy is an exclusion criterion and women of childbearing potential must not be considering getting pregnant during the study. Patients of reproductive potential must practice an effective method of contraception during treatment and for 6 months following the last rucaparib dose. No waivers of these inclusion or exclusion criteria will be granted by the investigator and the sponsor or its designee for any patient enrolled into the study.
Study Treatment	Patients will take 600 mg rucaparib orally twice daily (BID; as close to 12 hours apart as possible, preferably at the same times every day) with at least 8 oz (240 mL) of water starting on Day 1. Rucaparib may be taken with an empty stomach or with food. Rucaparib will be provided as 60, 120, 200, and 300 mg [as free base] dose strength tablets. Patients will take rucaparib BID for continuous 28-day cycles until disease progression as assessed by the investigator, or other reason for discontinuation. Dose reductions are permitted in the event of unacceptable toxicity.
Interim Safety Monitoring	A formal safety data review will occur after the first 20 patients have been enrolled, then quarterly until Part 1 of the study is fully enrolled, and then every 6 months thereafter. The review committee will include external experts and Sponsor personnel. The protocol will be amended as appropriate to incorporate additional patient safety monitoring if new safety signals are noted at any review. In the event that the recommended Phase 2 dose of 600 mg BID rucaparib is determined to be unsuitable for chronic dosing, the starting dose may be decreased to Dose Level -1 (480 mg / 500 mg BID rucaparib) for all subsequent patients if agreed upon between the Sponsor and the Principal Investigators.
Withdrawal Criteria	A patient must be discontinued from protocol-prescribed therapy if <u>any</u> of the following apply: • Consent withdrawal at the patient's own request or at the request of their legally authorized representative • Progression of patient's underlying cancer • Any event, adverse or otherwise, that, in the opinion of the investigator, would pose an unacceptable safety risk to the patient • An intercurrent illness that, in the opinion of the investigator, would affect assessments of the clinical status to a significant degree and requires discontinuation of therapy • A positive pregnancy test at any time during the study
Disease Assessments for Efficacy	Efficacy measures will include tumor assessments using computed tomography (CT) scans of the chest, abdomen, and pelvis with appropriate slice thickness per RECIST, CA-125 measurement, and clinical examination; other studies (magnetic resonance imaging [MRI], X-ray, positron emission tomography [PET], and ultrasound) may be performed if required. Disease assessments will

<table>
<tr><td>Disease Assessments for Efficacy (cont)</td><td>be performed at screening, at the end of every 8 weeks (±4 days) during the treatment and post-treatment (if patient discontinued treatment for any reason other than radiologically confirmed disease progression) phases until radiologically confirmed disease progression, death or initiation of subsequent treatment. Disease assessments should also be done at the time of treatment discontinuation if it has been ≥8 weeks since the last assessment. Patients who have been on study at least 18 months, may decrease the frequency of tumor assessments to every 16 (±2) weeks. If a complete response (CR) or partial response (PR) is noted, confirmatory scans should be performed at least 4 weeks after response was first documented. CA-125 <ULN will be required to designate a CR.

Copies of CT scans will be collected from all patients in Part 2 of the study and may be collected from selected patients in Part 1 of the study. Independent radiology review may be conducted on all or a subset of CT scans.</td></tr>
<tr><td>Statistical Procedures</td><td>Sample Size Justification

Part 1: Approximately 180 patients will be enrolled in order to ensure each HRD subgroup, tBRCA (HRD related to a deleterious BRCA1 or BRCA2 gene mutation in tumor tissue), nbHRD (no BRCA1 or BRCA2 mutation; LOH^{+}) and biomarker negative (no <i>BRCA1</i> or <i>BRCA2</i> mutation; LOH^{-}), will contain an adequate number of patients. Other than the cap on known gBRCA patients (n=15), there will be no specific requirement to enroll defined numbers of patients into each planned subgroup. The likely size of each subgroup has been estimated based on: a) frequencies of HRD-associated abnormalities at initial diagnosis as reported in the literature and b) the hypothesis that the inclusion criterion of sensitivity to platinum following the most recent line of platinum therapy will enrich the population for patients with tumors harboring mutations of HRD pathway genes (i.e., the frequency will be greater than that described in the newly-diagnosed population). The table below provides estimates for HRD subgroup sizes in Part 1 of this trial.

Estimated HRD Subgroup Sizes

<table>
<tr><th>HRD Subgroup</th><th>Expected Frequency at Diagnosis[a]</th><th>Estimated Frequency with Enrichment for Platinum Sensitivity</th><th>Estimated Number of Patients</th></tr>
<tr><td>tBRCA</td><td>21%</td><td>30%</td><td>15 with known deleterious gBRCA mutation (fixed)
plus
20 – 25 with sBRCA mutation
plus
5 – 25 with newly diagnosed gBRCA mutation</td></tr>
<tr><td>nbHRD</td><td>22 – 32%</td><td>30 – 50%</td><td>50 – 90</td></tr>
<tr><td>Biomarker Negative</td><td>60 – 70%</td><td>20 – 40%</td><td>36 – 72</td></tr>
<tr><td colspan="4">[a]Expected frequency estimates are from TCGA[1]</td></tr>
</table>
Enrollment of patients known a priori to harbor a gBRCA mutation classified</td></tr>
</table>

Statistical Procedures (cont)

as deleterious (pathogenic), suspected deleterious, or favor deleterious (or the equivalent interpretation of any of these) on the most recent assessment by a testing laboratory will be limited to 15 in Part 1. Fifteen patients with a known *gBRCA* mutation are sufficient to establish that the frequency of *gBRCA* mutation reversions is low. If none of the patients with a known *gBRCA* mutation is shown to have a reversion between archival tissue and tumor tissue collected at screening, then the frequency of *gBRCA* reversions is likely less than 20% as the upper bound of the 90% confidence interval (CI) is 18%. Additional patients may be identified as having a deleterious *BRCA* mutation in tumor tissue, therefore the tBRCA subgroup will likely contain at least 40 patients.

The benefit of rucaparib is expected to be the greatest in patients in the tBRCA subgroup, followed by patients in the nbHRD subgroup, and lowest in patients in the biomarker negative subgroup. This study will provide evidence as to whether the benefit of rucaparib is clinically meaningful in each of these subgroups, and particularly in the nbHRD subgroup.

With a total of 180 patients enrolled in Part 1 of the study, the comparison of any 2 subgroups will likely contain about 100 patients. Therefore with 100 patients, there is 80% power at a 2-sided 10% significance level to detect a difference in PFS distributions assuming the hazard ratio between 2 subgroups is 0.50.

Part 2: The objective of Part 2 is to estimate the ORR in each of the HRD subgroups in a heavily pre-treated patient population (at least 3, and and no more than 4, prior chemotherapy regimens). Up to 300 patients will be enrolled in Part 2 of the study in order to enroll at least 80 patients in each HRD subgroup. A total of 300 patients should be sufficient assuming an approximate 33.3% allocation to each HRD subgroup in the enrollment population.

Currently, there are few clinical studies that have prospectively evaluated response to treatment beyond the 3rd-line setting; however, retrospective analysis of patients in 3rd relapse and beyond indicate they have a short PFS (approximately 4-6 months) and OS (approximately 5-6 months).[23] Overall, there is a need for new treatments and alternatives to chemotherapy for heavily pre-treated ovarian cancer patients with advanced, relapsed disease to be explored in prospectively designed trials.

The table below provides 95% CIs for observed response rates ranging from 10 to 60% assuming a total of 80 patients within each HRD subgroup.

Confidence Intervals for Objective Response Rates (ORR)

ORR(%)	[95% CI]
10	4.4, 18.8
20	11.8, 30.4
30	20.3,41.3
40	29.2,51.6
50	38.6, 61.4
60	48.4, 70.8

CI=Confidence intervals of ORR using Clopper-Pearson methodology.[24]

Statistical Procedures (cont'd)	An ORR ≥20% in any subgroup would be worthy of further exploration in that population. **Efficacy Analyses** Kaplan-Meier methodology will be used to summarize time to event variables. Response will be determined using Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1 and Gynecologic Cancer InterGroup (GCIG) CA-125 criteria. The overall response rate will be summarized with frequencies and percentages. The duration of response will be summarized with descriptive statistics (N, mean, standard deviation, median, minimum, and maximum) as well as categorically. The efficacy analyses will be evaluated for all patients treated in the study as well as for each of the HRD subgroups. **Safety Analyses** Adverse events (AEs), clinical laboratory results, vital signs, ECOG performance status, body weight, and concomitant medications/procedures will be tabulated and summarized. AEs will be summarized overall and separately for serious AEs, AEs leading to discontinuation, AEs leading to death, and NCI CTCAE Version 4.0 Grade 3 or higher AEs. Body weight and vital signs will be summarized descriptively (N, mean, standard deviation, median, minimum, and maximum). ECOG will be summarized categorically.

2 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AAG	alpha-1 acid glycoprotein
ADP	adenosine diphosphate
AE	adverse event
AIDS	acquired immunodeficiency syndrome
ALP	alkaline phosphatase
ALT	alanine transaminase
ANC	absolute neutrophil count
AST	aspartate transaminase
AUC	area under the curve
BER	base excision repair
BID	twice a day
BMT	bone marrow transplant
BRCA1	breast cancer susceptibility gene 1
BRCA2	breast cancer susceptibility gene 2
$BRCA^{mut}$	*BRCA1* and/or *BRCA2* gene(s) harboring a deleterious mutation
$BRCA^{unk}$	*BRCA1* and *BRCA2* genes with unknown mutation status
$BRCA^{wt}$	wild-type *BRCA1* and *BRCA2* gene sequences
BUN	blood urea nitrogen
CA-125	cancer antigen 125
ctDNA	circulating cell-free tumor DNA
CFR	Code of Federal Regulations
CI	confidence interval
CK	creatinine kinase
C_{max}	maximum concentration
CNS	central nervous system
CPK	creatine phosphokinase
CR	complete response
CRO	contract research organization
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events (version 4.0)
CYP	cytochrome P450
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DOR	duration of response
DSB	double-strand break
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EDC	electronic data capture

EOC	epithelial ovarian cancer
EOS	end of study
EOT	end of treatment
FFPE	formalin-fixed paraffin-embedded
GALT	gut-associated-lymphoid tissue
gBRCA	germline *BRCA*
GCIG	Gynecologic Cancer InterGroup
GCP	Good Clinical Practice
GGT	gamma glutamyl transferase
GS	genomic scarring
h	hour
H & E	hematoxylin and eosin
HDL	high-density lipoprotein
hERG	human ether-a-go-go-related gene
HGSOC	high-grade serous ovarian cancer
HIPAA	Health Information Portability and Accountability Act
HIV	human immunodeficiency virus
HR	hazard ratio
HRR	homologous recombination repair
HRD	homologous recombination deficiency
HNSTD	highest non-severely toxic dose
IC_{xx}	concentration where maximum response is inhibited by XX%
IEC	Independent Ethics Committee
IHC	immunohistochemistry
INR	international normalized ratio
IRB	Institutional Review Board
irr	independent radiology review
irrORR	objective response rate assessed by independent radiology review
IVRS/IWRS	Interactive Voice Response System/Interactive Web Response System
LDL	low-density lipoprotein
LOH	loss of heterozygosity
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Drug Regulatory Activities
Min	minute
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
nbHRD	non-BRCA homologous recombination deficiency
NCCN-FACT	National Comprehensive Cancer Network-Functional Assessment of Cancer
NCI	National Cancer Institute

NGS	next generation sequencing
NHEJ	non-homologous end-joining
NOAEL	no-observed-adverse-effect level
ORR	objective response rate
OS	overall survival
PARP	poly(adenosine diphosphate [ADP]-ribose) polymerase
PBL	peripheral blood lymphocytes
PD	progressive disease
PET	positron emission tomography
PLD	PEGylated liposomal doxorubicin
PFS	progression-free survival
P-gp	P-glycoprotein
PK	pharmacokinetic(s)
PR	partial response
PT	prothrombin time
QD	once a day
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	recommended Phase II dose
SAE	serious adverse event
SAP	statistical analysis plan
SAS	statistical analysis software
SD	stable disease and standard deviation
SI	international units
SNP	single-nucleotide polymorphism
SOC	system organ class
SSB	single-strand break
STD	severely toxic dose
SUSAR	suspected unexpected serious adverse reaction
TAm-Seq	tagged-amplicon deep sequencing
$t_{1/2}$	half-life
TCGA	The Cancer Genome Atlas
TEAE	treatment-emergent adverse event
T_{max}	time to maximum concentration
TMZ	temozolomide
TP53	tumor protein p53
ULN	upper limit of normal
UV	ultraviolet
WBC	white blood cell
WOCBP	women of childbearing potential
wt	wild-type

3 INTRODUCTION

3.1 Ovarian Cancer

3.1.1 General Overview

Ovarian cancer is the second most common gynecologic malignancy worldwide and the leading cause of death attributed to gynecological cancer.[25,26] After initial therapy, most women will have a progression-free interval of approximately 1.5 to 2 years, depending on the extent of post-operative residual disease and response to chemotherapy.[27] Relapse still occurs, however, in the majority of cases, and only 10–30% of women experience long-term survival.[27] Advanced stage disease is associated with a 5-year survival rate of only 30–40%.[25]

Approximately 90% of ovarian tumors are surface epithelial in origin, and the papillary serous histology subtype accounts for approximately 75%, of which the large majority (70%) is high-grade.[27] The site of origin of epithelial ovarian cancer remains unclear. Some studies suggest that serous epithelial ovarian cancer (EOC) and primary peritoneal cancer (PPC) arise from the fallopian tube epithelium; however, other studies suggest an origin within stem cells of the ovarian surface epithelium.[27-31] EOC, PPC and fallopian tube cancer behave very similarly and are therefore treated in the same way.

The median age at presentation of EOC is 60 years. Due to the non-specific nature of symptoms, many women present with advanced disease and therefore have a poor prognosis.

3.1.2 Treatment of Ovarian Cancer

The standard approach to treatment of advanced high-grade serous ovarian cancer (HGSOC) is cytoreductive surgery (either at time of diagnosis or interval debulking), with the goal of minimizing residual tumor to no visible residual disease, a major prognostic indicator for improved survival. Six to eight cycles of platinum- and taxane-based chemotherapy is the global standard of care. If initial cytoreduction is not performed, interval debulking surgery is considered. This surgery may be carried out after three or four cycles of primary chemotherapy, followed by three further cycles of chemotherapy. Platinum analogues, such as carboplatin and cisplatin, are the most active agents, mediating their effects through the formation of inter- and intra-strand cross-links with deoxyribonucleic acid (DNA).[27,32]

The choice of treatment for relapsed disease is based on the treatment-free interval relative to last therapy administered and chemotherapy agents used. Platinum-based regimens dominate ovarian cancer therapy and define treatment groups.[33] In general, patients whose disease progresses during treatment with a platinum-based regimen are considered to have platinum-refractory disease; patients whose disease relapses within 6 months after the last platinum agent was administered are considered to have platinum-resistant disease; and patients whose disease relapses more than 6 months after last platinum-based therapy was administered are considered to have platinum-sensitive disease. However, these classifications are somewhat arbitrary as resistance to platinum-based therapy is a time continuum, not a categorical variable, and a status of ‘platinum-resistant’ is not absolute as it can be partially overcome. In addition, ‘platinum-sensitivity’ was defined when there was no alternative to platinum-based treatment and in

clinical practice typically only refers to second-line treatment. These definitions also do not take into account the molecular characteristics of a patient's tumor (i.e. HRD such as BRCA mutations). In later lines of therapy, treatment choice is often restricted according to the invidual patient situation (e.g. performance status, organ function, residual toxicities from prior treatment, other comorbidities, and patient choice).

As many patients experience multiple relapses, prognosis and response to therapy decreases as the interval between last chemotherapy exposure and disease relapse shortens. The treatment-free, or specifically the platinum-free interval, provides further prognostic information for patients, as therapeutic options lessen and survival shortens as a patient's tumor becomes less responsive to platinum-based therapy. Patients who have received several prior lines of treatment are known to have strongly dimished treatment-free intervals and response rates and the benefits of continued treatment with conventional chemotherapy often does not outweigh the risk of additional toxicity. This patient population is a group with limited treatment options that could benefit from treatment with a targeted agent that takes the molecular characteristics of their disease into account.[23,34]

3.1.3 Role of BRCA and HRD in Ovarian Cancer

DNA is constantly damaged by both endogenous and exogenous (environmental) assaults. A common type of DNA damage is the formation of DNA single-strand breaks (SSBs). During normal cell cycling, DNA is replicated and replication forks are eventually stalled by persistent SSBs. If stalled replication forks are not rapidly repaired, they can often degenerate and form DNA double-strand breaks (DSBs), which are highly likely to be lethal to the cell.

Single-strand breaks are normally quickly repaired by a process known as base excision repair (BER). The BER process is initiated by the activity of the poly (adenosine diphosphate [ADP]-ribose) polymerase (PARP) enzyme. In normal cells, an additional DNA repair process known as homologous recombination repair (HRR) can repair DSBs. Homologous recombination is a complex, multistep process, in which two key components are the proteins encoded by the breast cancer susceptibility 1 and 2 genes (*BRCA1* and *BRCA2*).

Germline mutations in *BRCA1* and *BRCA2* genes are the strongest known hereditary factors for breast and EOC, accounting for up to 5% of all breast cancers and 15% of all EOCs.[2,3] These patients carry heterozygous deleterious mutations in their germline DNA, and develop tumors when the remaining wild-type functional allele is inactivated (i.e., "second hit"). Approximately 6 – 8% of patients with HGSOC have somatic mutations in *BRCA1* or *BRCA2*.[1,4]

If either the BER or HRR pathway is rendered non-functional, the remaining functional pathway can compensate to ensure ongoing DNA repair and cell cycling. For example, when the BRCA-associated HRR pathway is lost or dysfunctional, repair shifts towards the BER repair pathway that is dependent on PARP enzymes. In contrast, in the setting in which both repair pathways (BER and HRR) are rendered non-functional, the cell dies. This concept, where a defect in either of two pathways can be withstood by a cell, but defects in both are lethal, is referred to as synthetic lethality. This type of lethality can arise from a variety of different interactions. In the case of DNA damage repair, this state of dual non-functionality can be

achieved by enzymatic inhibition of PARP in the context of a genetic mutation in the *BRCA1* or *BRCA2* genes.

Two key *in vitro* and *in vivo* studies demonstrated the concept of synthetic lethality in DNA repair. Bryant and colleagues showed that cell lines and a tumor xenograft deficient in *BRCA2* were highly sensitive to PARP inhibition.[5] In a parallel set of experiments, Farmer and colleagues illustrated that chemical inhibition of PARP-1 was more potent in homozygous *BRCA1/2*-deficient cell lines than in heterozygous mutant or wild-type cell lines.[6] These findings were also supported by a *BRCA2*-deficient murine model. Taken together, these studies provide support for the treatment of patients with a *BRCA*-deficient tumor with a PARP inhibitor.

However, defects in the HRR pathway are not limited solely to mutations of *BRCA1*/2. Genetic alterations of many different HRR pathway genes are associated with human cancers, with the percentage of tumors affected by homologous recombination deficiency (HRD) varying considerably across different tumor types. The Cancer Genome Atlas (TCGA), which completed an analysis of molecular changes in HGSOC, estimated that approximately 50% of patients with HGSOC have alterations in genes involved in the HRR DNA repair.[1] Of those, approximately 27% are estimated to have HRD due to a gene mutation or other genomic alteration or modification (e.g., epigenetic silencing) that is not associated with a *BRCA1/2* mutation.[1] Approximately 15% of patients are estimated to have a gene mutation in a HRR pathway gene other than *BRCA1/2*.[1]

An alternative approach in identifying non-*BRCA* patients with HRD is to detect genomic scars within the tumor, which arise from the use of error-prone DNA repair pathways when HRR is compromised.[35,36] Through a series of experiments and data analyses, the Sponsor has determined that a potential method for identifying patients who may be sensitive to rucaparib is to assess genomic scarring by quantifying the extent of loss of heterozygosity across the tumor genome (tumor genomic LOH). One of the main advantages of detecting tumor genomic LOH is that it can identify HRD tumors regardless of the underlying mechanisms, which include both known (i.e., *BRCA* mutations) and unknown genomic mechanisms.[37,38]

3.2 PARP Inhibitors

PARP inhibitors have been evaluated in the clinic for the past decade. Iniparib (BSI-201) was initially the furthest advanced, with a Phase 3 randomized study in combination with gemcitabine and carboplatin conducted in patients with triple-negative metastatic breast cancer. Data from this study showed that patients receiving iniparib with chemotherapy did not experience significant improvements in overall survival (OS) or progression free survival (PFS) compared to patients receiving just the chemotherapy regimen.[39] Since then, several groups have determined that the primary mechanism of action for iniparib is not via inhibition of PARP activity.[40,41]

Rucaparib has demonstrated compelling activity in ovarian cancer patients with a *BRCA* mutation as well as in patients without a *BRCA* mutation (see Section 3.3). Durable responses have been observed in both platinum-sensitive and platinum-resistant disease.

Olaparib (AZD-2281), another investigational PARP inhibitor, has also demonstrated Phase 2 clinical activity, both in treatment and maintenance settings, in metastatic breast cancer patients with a germline *BRCA* (*gBRCA)* mutation and in relapsed HGSOC patients (both *BRCA* mutant and wild-type). The concept of synthetic lethality was exploited in two proof-of-concept clinical studies with olaparib in patients with *BRCA*-associated tumor types. These studies evaluated the efficacy and safety of continuous oral dosing with olaparib in women with either relapsed ovarian cancer or advanced breast cancer and included women with and without a *gBRCA* mutation.[8,9] In these patients, who had received a median of three prior chemotherapy regimens, encouraging overall response rates of 33% and 41%, were observed, in ovarian and breast cancer, respectively. In a third study, olaparib treatment was associated with a greater overall response rate (ORR) in patients with *gBRCA*-associated ovarian cancer compared with the patients in the non-*BRCA* associated cohort (41% vs 24%, respectively).[10] In a fourth study that evaluated olaparib versus PEGylated liposomal doxorubicin (PLD) in patients with a *gBRCA* mutation and relapsed ovarian cancer, the efficacy of olaparib was consistent with that observed in previous studies.[42] More recently, olaparib demonstrated good clinical activity (31% ORR) in *gBRCA*^{mut} ovarian cancer patients (n=193) with platinum-resistant disease who received a mean of 4.3 prior treatment regimens.[43]

Activity in HGSOC has also been observed with switch maintenance therapy following response to platinum-based chemotherapy.[44] Patients with platinum-sensitive relapsed ovarian cancer who achieved a response to another regimen of platinum-based chemotherapy followed by olaparib as switch maintenance treatment experienced a statistically significant improvement in median PFS (8.3 months) compared to patients who received placebo as maintenance therapy (4.8 months); hazard ratio of 0.35 (95% CI, 0.25 – 0.49).[44] Patients with a *BRCA* mutation derived the most benefit (median PFS 11.2 vs 4.3 months; HR, 0.18; 95% CI 0.11-0.31; $P<0.00001$).[14] It should be noted that outcomes were the same in patients who had a *gBRCA* mutation and those who had a somatic *BRCA* (*sBRCA*) mutation, suggesting that it is appropriate to not differentiate between germline and somatic mutations. Patients without a *BRCA* mutation also experienced significant benefit from treatment with olaparib (HR=0.53; 95% CI 0.33-0.84; $P=0.007$).[14]

Niraparib (MK-4827), another PARP inhibitor with a similar mechanism of action to olaparib, exhibited clinical activity in both *BRCA*-mutated ovarian cancer (8 RECIST PRs) and sporadic ovarian cancer (2 RECIST PRs and/or GCIG CA-125 responses) patients in a Phase 1 study.[45,46] In a Phase 1 evaluation of BMN 673, also a PARP inhibitor, 11 of 17 *BRCA*-mutated ovarian cancer patients treated at doses ≥100 µg/day exhibited a RECIST and/or CA-125 response.[47]

It is worth noting that PARP inhibitor monotherapy has elicited objective responses in patients with platinum-sensitive disease as well as in patients with platinum-resistant disease, although response rates are higher in the former population.[10,42,46] This indicates that using platinum-sensitivity alone as a selection marker for PARP inhibitor therapy is not an effective tool.

These data support the potential role for the PARP inhibitor rucaparib in the treatment of patients with *BRCA*-associated ovarian cancer. Furthermore, the 24% ORR in the non-BRCA cohort described above and the benefit of maintenance PARP inhibitor treatment in patients without a *BRCA* mutation suggest that the clinical utility of PARP inhibitors can be extended to a larger patient group with HRD based on HRR alterations other than *BRCA*, i.e., nbHRD.[10,14]

Assessing tumor genomic LOH in this trial provides a mechanism to identify patients with HRR alterations who may benefit from treatment with rucaparib but do not harbor a deleterious *BRCA1* or *BRCA2* mutation.

Emerging data with PARP inhibitors also support evaluation of rucaparib in relapsed ovarian cancer patients with advanced disease who have received multiple prior lines of treatment, a patient population for whom there are limited treatment options currently.

3.3 Rucaparib

Rucaparib (CO-338; formerly known as PF-01367338 and AG-014447) is an orally available, small molecule inhibitor of PARP-1 and PARP-2. Rucaparib is specific for PARP-1 and PARP-2 based on results of direct biochemical assays and an off-target receptor panel. Nonclinical evaluation has demonstrated exquisite sensitivity of *BRCA1* and *BRCA2* homozygous mutant cell lines to rucaparib and provides a rationale for the clinical assessment of rucaparib as monotherapy in patients with hereditary deficiencies of *BRCA1* and/or *BRCA2*. Rucaparib has also shown antitumor activity as a single agent in the MDA-MB-436 (*BRCA1* mutant) xenograft mouse model.

The details of these and other nonclinical experiments are provided in the Investigator's Brochure.

3.3.1 Nonclinical Experience

3.3.1.1 Rucaparib Absorption, Distribution, Metabolism, and Excretion

The pharmacokinetics (PK) and toxicokinetics of rucaparib camsylate following oral administration, the intended route of administration in humans, was evaluated in the mouse, rat, and dog. The time at which the peak plasma concentrations were observed (T_{max}) occurred at 1–3 hours post dose in the mouse and dog, with the rat generally exhibiting a later T_{max} (4–8 hours). The oral bioavailability was 17%, 36%, and 62%, respectively, in the mouse (50 mg/kg), rat (100 mg/kg), and dog (20 mg/kg). In the rat and dog, there were no marked gender-related differences and no accumulation after repeat oral administration. A less than dose-proportional increase in exposure was observed in the rat and dog when rucaparib was administered as a suspension in 0.5% methylcellulose; however, a greater than dose-proportional increase in exposure was observed in the 1-month dog toxicity study when rucaparib was administered in capsules.

Rucaparib PK, following IV administration of salts of rucaparib, were evaluated in mice, rats, dogs, and monkeys. IV dosing with the glucuronate or phosphate salt of rucaparib resulted in moderate to rapid clearance and a large volume of distribution, indicating this compound is well distributed in the body. The half-life ($t_{1/2}$) ranged from 2.3 to 5.2 hours.

In vitro plasma protein binding studies in mouse, rat, and dog plasma showed moderate binding and ranged from 49.5% to 73%. Plasma protein binding in humans ranged from 55% to 75%.

Recombinant cytochrome P450 (CYP) studies indicated that CYP2D6, CYP1A2, and to a lesser extent, CYP3A4, have the ability to metabolize rucaparib. Rucaparib moderately inhibited CYP1A2, CYP2C19, and CYP2C8. In addition, rucaparib showed mixed inhibition of CYP2C9. Based on bi-directional experiments of digoxin transport carried out using Caco-2 cells, it was determined that rucaparib is a moderate P-glycoprotein (P-gp) inhibitor. The inhibition potential for rucaparib on P-gp will likely be low at clinical oral doses ≤200 mg. At doses >200 mg, patients taking digoxin should have their digoxin levels monitored regularly via standard clinical practice.

Quantitative whole body autoradiography studies in Long-Evans rats showed [^{14}C] rucaparib radioequivalents were rapidly and widely distributed to tissues following IV administration, consistent with a large volume of distribution. At 2 minutes after dosing, highest concentrations were found in kidney, lung, thyroid gland, heart, stomach mucosa, liver adrenal glands, spleen, and blood. Little radioactivity was present in brain; levels were undetectable at 15 minutes after dosing. Activity was undetectable in most tissues by 96 hours after dosing, however levels in the choroid/retina declined more slowly, and persistent radioactivity was also found in hair follicles through 192 hours, indicating that drug equivalents have high affinity and long half-life in pigmented tissues. High levels of radioactivity were observed in ureters, bladder, and bile ducts, indicating both renal and biliary routes eliminated drug equivalents.

3.3.1.2 Multiple-Dose Toxicity Studies

Rucaparib was evaluated in both rat and dog in oral and IV infusion toxicity studies. Only the multiple-dose toxicity studies utilizing the oral formulation are summarized below. Details of these studies are provided in the Investigator's Brochure.

Target organs identified in studies where rucaparib was administered orally include the hematopoietic system and gastrointestinal tract. No cardiovascular findings were noted in any of the oral toxicity studies.

Multiple-Dose Oral Toxicity Studies in Rats

Administration of rucaparib camsylate salt via oral gavage was generally well-tolerated in the rat up to 1000 mg/kg/day for 7 days and up to 150 mg/kg/day for 28 days. Decreases in body weight gain and food consumption were noted in both studies. In the 7-day study, target organs identified microscopically were bone marrow, spleen, and thymus. Minimal to mild bone marrow hypocellularity was noted in all dose groups. The no-observed-adverse-effect-level (NOAEL) was established at 500 mg/kg/day.

In the 28-day study, there were 3 rucaparib-related deaths at 500 mg/kg/day immediately after blood collection on Day 28 (n=1) or Day 29 (n=2; first day of recovery phase). These deaths likely resulted from the marked anemia identified hematologically. Other rucaparib-related clinical signs at 500 mg/kg/day included thinning haircoat and pale eyes. Identified target organs included bone marrow, spleen, lymphoid tissue (thymus, gut-associated-lymphoid tissue [GALT], and lymph nodes), and cecum (at 500 mg/kg/day only). Following cessation of rucaparib dosing, most findings reversed. In this study, the severely toxic dose in 10% of the animals (STD10) was 500 mg/kg/day and the NOAEL was 50 mg/kg/day.

Rucaparib camsylate in capsules was also given orally to rats at doses of 10, 40, and 100 mg/kg/day for 91 consecutive days with a 28-day recovery period. Decreased body weight and body weight gain were observed for animals given ≥40 mg/kg/day. At the end of the recovery phase, mean body weight was still lower for males given 100 mg/kg/day and females given ≥40 mg/kg/day. Hematological findings included decreases in red blood cell mass parameters in animals given ≥40 mg/kg/day (which correlated with decreased bone marrow hypocellularity), and decreases in reticulocytes, white blood cells (WBC) and absolute lymphocytes at ≥40 mg/kg/day. The latter changes correlated with the microscopic findings of decreased lymphocytes in the mandibular lymph nodes and gut-associated lymphoid tissue. All effects were reversible. Microscopically, bone marrow hypocellularity at 100 mg/kg/day and minimally decreased lymphocytes in lymphoid tissues at ≥40 mg/kg/day were noted and were completely reversed at the end of the recovery period. The NOAEL was established to be 100 mg/kg/day.

Multiple-Dose Oral Toxicity Studies in Dogs

Oral gavage administration of the camsylate salt form of rucaparib to dogs for 7 days resulted in gastrointestinal clinical signs at the 80 mg/kg/day high-dose group. Hematopoietic effects of decreased reticulocytes were noted in mid- to high-dose groups and leukopenia was exhibited in all treatment groups. Lymphoid atrophy occurred in both sexes and in all treatment groups. Decreased bone marrow cellularity was seen in both sexes (males at all doses; females at 80 mg/kg/day). A 7-day repeat-dose toxicity study using oral capsules in dogs was repeated in order to characterize the toxicity of a new lot of rucaparib camsylate. Similar to the results of the prior 7-day study in dog, gastrointestinal clinical findings were noted at 80 mg/kg/day. Vomiting was observed throughout the dosing phase for males as well as liquid and/or mucoid feces in both genders. Decreased food consumption was observed at 80 mg/kg/day that correlated with body weight loss that was considered adverse. Decreases in erythroid, platelet, and leukocyte parameters were observed primarily at 80 mg/kg/day and occasionally at 20 or 5 mg/kg/day. These data indicated that the drug targeted multiple bone marrow lineages in a dose-related pattern.

Rucaparib camsylate salt in capsules was administered orally to dogs for 30 consecutive days with a 29-day recovery. Gastrointestinal clinical signs were noted at ≥ 5 mg/kg/day, with decrease in food consumption at 75 mg/kg/day. Adverse hematological changes (decrease in erythroid, myeloid, and megokaryocytic lineages) occurred at ≥ 20 mg/kg/day. Effects were fully reversible. The NOAEL in this study was 5 mg/kg/day.

Rucaparib camsylate in capsules was also given orally to dogs at doses of 3, 15/10, 40/30/20 mg/kg/day for 91 consecutive days with a 29-day recovery period. Body weight losses and inappetance observed at the high dose in both sexes during the first quarter of the dosing phase were considered adverse and resulted in dose reductions (40 to 30 to 20 mg/kg/day for toxicity and 15 to 10 mg/kg day in order to maintain multiples of exposures for optimal testing of dose response) for the remainder of the study. Clinical pathology findings were indicative of bone marrow toxicity; these changes were nonprogressive over time suggesting potential adaptation to these initial effects. Hematological findings at 40/30/20 mg/kg/day correlated with erythroid atrophy of the bone marrow detected microscopically. By Day 29 of recovery, most effects reversed. The highest non-severely toxic dose (HNSTD) for this study was 20 mg/kg/day

for male dogs. No HNSTD was established for female dogs. The NOAEL was 10 and 20 mg/kg/day for male and female dogs, respectively.

3.3.1.3 Additional Observations

In vitro genetic toxicology assays demonstrated oral rucaparib to be clastogenic. Bacterial mutagenicity data for rucaparib were clearly negative in four microbial tester strains, both with and without metabolic activation, and equivocal in a fifth tester strain.

In an in vitro assay for human ether-a-go-go-related gene (hERG) activity, the IC_{50} and IC_{20} for the inhibitory effects of rucaparib (50% inhibitory concentration and 20% inhibitory concentration) on hERG potassium currents were 24 μM (7761 ng/mL) and 7 μM (2264 ng/mL), respectively. These values are 7-fold and 2-fold higher, respectively, than the highest (unbound) steady state plasma concentrations observed to date in humans (3710 ng/mL x 0.298 F_u = 1106 ng/mL) at a dose of 600 mg BID rucaparib administered orally.

Effects on appearance and behavior, motor activity, body temperature, and a number of neurofunctional tests and reflexes were evaluated in rats. A dose of 50 mg/kg of rucaparib administered via IV infusion (mean C_{max}=13629 ng/mL) resulted in a significant reduction in motor activity compared with vehicle-treated animals; however, there were no effects on neurofunctional or reflex testing at this dose. The plasma concentration measured at this dose is 3.7-fold above the highest steady state plasma concentration (3710 ng/mL) observed to date in humans at a dose of 600 mg BID rucaparib administered orally.

Administration of rucaparib to Long-Evans rats orally at doses up to 750 mg/kg/dose, followed by a single exposure to solar-simulated ultraviolet radiation approximately 4 hours after the final dose elicited no skin or ocular reactions indicative of phototoxicity. The no-observed-effect-level (NOEL) for phototoxicity was >750 mg/kg/day.

Additional information may be found in the current Investigator's Brochure.

3.3.2 Clinical Experience

The early clinical program assessed safety and efficacy in patients with malignancies commonly treated with chemotherapeutic agents, initially with the IV formulation of rucaparib administered in combination with a variety of chemotherapies, and later with the oral formulation of rucaparib administered as a monotherapy. The latter is the focus of current development efforts.

3.3.2.1 Rucaparib Monotherapy

Rucaparib monotherapy is currently being evaluated as treatment for relapsed ovarian cancer in two Clovis-sponsored clinical studies (Study CO-338-010 and this study, CO-338-017 [ARIEL2]. Over 200 patients have been treated with the oral formulation of monotherapy rucaparib in open-label trials; over 150 patients have been treated with the recommended Phase 2 dose of 600 mg BID.

Study CO-338-010

Study CO-338-010 is a 2-part, open-label, safety, PK, and preliminary efficacy study of oral rucaparib administered daily for continuous 21-day cycles. Part 1 was a Phase 1 portion in patients with any solid tumor, including lymphoma, who have progressed on standard treatment. The primary objective of this portion of the study was to determine the optimal monotherapy dose for orally administered rucaparib. Measurable disease was not required and tumor marker assessments are optional. Part 2 is the ongoing Phase 2 portion in patients (up to n=41) with platinum-sensitive relapsed ovarian cancer with evidence of a gBRCA mutation who have received at least 2, but no more than 4, prior regimens. The primary objective of this portion of the study is to assess the overall objective response rate by RECIST v1.1 in this ovarian cancer patient population.

Study CO-338-010 was initiated in Q4 2011. In the Phase 1 portion, a total of 56 patients (median age 50 years [range 21–71]; 51 female; 27 breast cancer, 20 ovarian/peritoneal cancer, 2 pancreatic cancer; 7 other tumor) were treated at dose levels of 40, 80, 160, 300, and 500 mg QD, and 240, 360, 480 and 600 mg BID rucaparib administered continuously. Two patients are still receiving treatment as of November 2014. One patient treated with 360 mg BID rucaparib experienced a dose-limiting toxicity (DLT) of Common Toxicity Criteria for Adverse Events (CTCAE) Grade 3 nausea despite maximal intervention in Cycle 1 of treatment. No DLTs were observed during Cycle 1 in the 480 mg BID and 600 mg BID cohorts however, similar to other PARP inhibitors, non-DLT myelosuppression was observed beyond Cycle 1, therefore the dose of 600 mg BID rucaparib was selected as the recommended dose for future Phase 2 and Phase 3 studies.

In the ongoing Phase 2 portion, 20 ovarian cancer patients (median age 56 [range 44-84]; ECOG performance status 0/1=12/8; median number of anticancer regimens=2 [range 2-4]; median number of platinum-based regimens=2 [range 2-3]) were enrolled as of September 2014.

Study CO-338-017 (ARIEL2)

In this ongoing trial, 143 ovarian cancer patients (median age 65 [range 31-86]; ECOG performance status 0/1/pending=95/47/1; median number of anticancer regimens=1 [range 1-6); median number of platinum-based regimens=1 [range 1-5]) have enrolled into Part 1 of the study as of October 2014. Full enrollment into Part 1 of the study is anticipated to be completed in December 2014.

3.3.2.1.1 Safety

As of November 2014, safety data are available for n=163 ovarian cancer patients treated with 600 mg BID rucaparib monotherapy in the ongoing Phase 2 studies, including Part 1 of this trial. Treatment-related adverse events (all grades) reported in ≥15% of patients treated with 600 mg BID rucaparib include: gastrointestinal and related symptoms (nausea, vomiting, dysgeusia, diarrhea, abdominal pain, and decreased appetite); anemia; fatigue/asthenia, and headache. Elevations of ALT and/or AST are also commonly observed. The ALT/AST elevations occur early (within first 2-4 weeks of treatment), are generally mild to moderate (Gr 1-2), are not accompanied by any changes in bilirubin levels, and often transient and resolved to within

normal ranges, or stabilize. No patient has met the laboratory criteria for Hy's Law.[48] As has been observed with rucaparib and other PARP inhibitors, myelosuppression may be delayed and observed after a period of continuous dosing. Grade 3/4 adverse events assessed as treatment-related and occurring in >5% of patients include: anemia/decreased hemoglobin and increased ALT. All treatment-related adverse events have been successfully managed with concomitant medications, supportive care, and treatment interruption and/or dose reduction. No patient has discontinued rucaparib treatment due to a treatment-related adverse event. A total of five patients have died on study or within 30 days of last dose of rucaparib; all deaths were due to disease progression and were assessed as not related to rucaparib.

Extensive centrally-reviewed electrocardiogram (ECG) monitoring was conducted in the Phase 1 portion of study CO-338-010. ECG results (as triplicate reads) are available for all 56 treated patients. No patient had a QTcF measurement ≥500 msec at any time during study participation. Only one patient had a QTcF measurement ≥480 msec. This measurement occurred in a patient receiving 480 mg BID rucaparib and concomitant administration of citalopram, a medication with known potential to cause QT prolongation. This patient has continued to receive monotherapy rucaparib at a dose of 480 mg BID with no further QTcF measurement ≥480 msec. No patient experienced a ≥60 msec increase in QTcF over baseline. A total of 11 patients experienced a QTcF increase ≥30 msec over baseline. Further analyses suggest a lack of relationship between QTcF increase ≥30 msec and dose or exposure. In addition, there were no adverse events suggestive of cardiac arrhythmia (e.g., presyncope, syncope, sudden death) in any patient. ECG and adverse event data to date in patients receiving monotherapy rucaparib at doses up to 840 mg BID suggest there is a minimal risk of QTc prolongation.

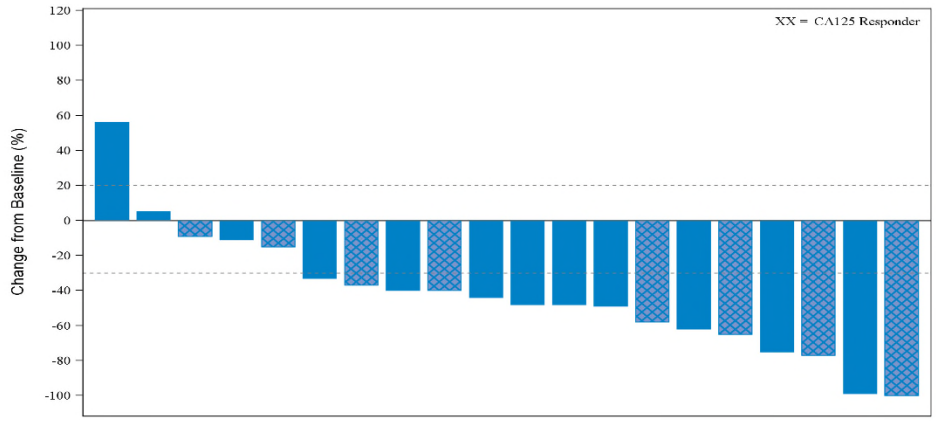
3.3.2.1.2 Efficacy

Study CO-338-010

In the Phase 1 portion, 2 patients (breast cancer and ovarian cancer, both *gBRCA*^{mut}) achieved a RECIST CRs and 7 patients (3 ovarian cancer, 4 breast cancer, 1 pancreatic cancer; all *gBRCA*^{mut}) achieved a RECIST PR during the dose escalation phase (n=2 at 300 mg QD; n=2 at 360 mg BID; n=3 at 480 mg BID; and n=2 at 600 mg BID). Response were durable across tumor types. In addition, 3 patients with ovarian cancer achieved a cancer antigen 125 (CA-125) response as defined by Gynecologic Cancer InterGroup (GCIG) criteria. The disease control rate (CR, PR, or SD>12 weeks) in evaluable ovarian cancer patients treated at doses ≥360 mg BID was 92% (11/12). Responses were observed in platinum-resistant as well as platinum-sensitive ovarian cancer patients. In platinum-resistant ovarian cancer patients treated with ≥360 mg BID rucaparib, 50% (4/8) achieved either a RECIST (25%, 2/8) or GCIG CA-125 response (25%, 2/8). The disease control rate (CR, PR, or SD>24 weeks) in this group was 75% (6/8) and median time on treatment was approximately 9 months (range 1.5-14.5).

In the Phase 2 portion of Study CO-338-010, compelling activity has been observed in patients who had received 2-4 prior chemotherapy regimens and a deleterious *BRCA* mutation, with 15 of 20 (75%) achieving a RECIST PR and 17 of 20 (85%) achieving a RECIST PR and/or a GCIG CA-125 response. The vast majority of patients had some level of target lesion measurement reduction as shown in Figure 1.

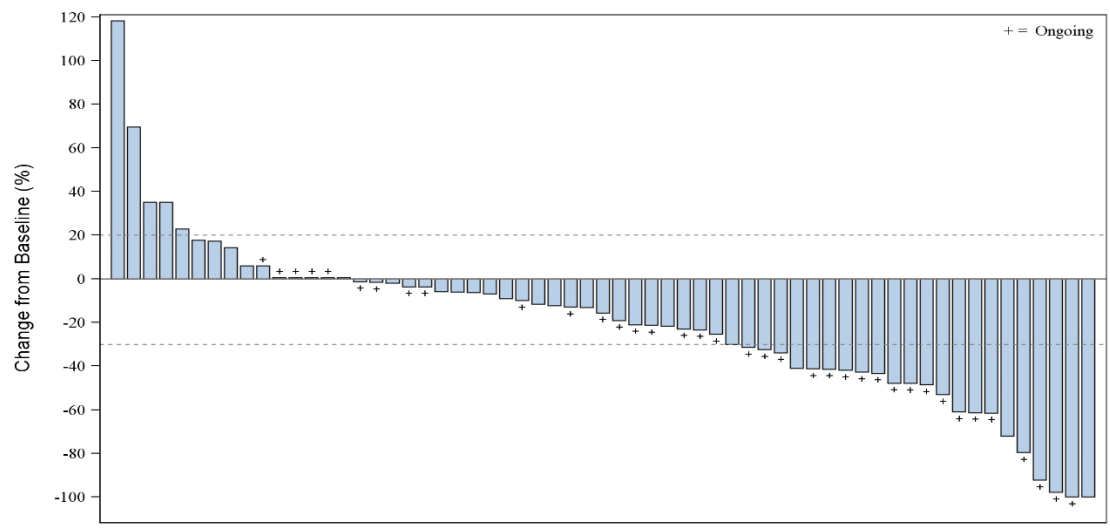
Figure 1 Best Target Lesion Response – Study CO-338-010 Phase 2



Study CO-338-017 (ARIEL2)

In Part 1 of this study, preliminary efficacy data as of October 2014 indicate target lesion reduction in the majority of patients currently evaluable for efficacy, as indicated in Figure 2. ORRs of 38% (RECIST) and 44% (RECIST & GCIG CA-125) have been observed in n=61 patients who had a baseline scan and at least one post-treatment scan, and who were able to be classified into 1 of 3 HRD subgroups based on analysis of their screening biopsy sample. The disease control rate (CR, PR or SD>24 weeks) in this group of patients is 78% and 61% of patients were continuing treatment with rucaparib at the time of this data analysis.

Figure 2 Best Target Lesion Response – Study CO-338-017 (ARIEL2) Part 1



Response and disease control rate data were also analyzed by HRD subgroup as shown in Table 1. Activity was enhanced, as expected, in the subset of patients with a BRCA mutation (n=23), with ORRs of 61% (RECIST) and 70% (RECIST and/or GCIG CA-125). Responses were observed in patients with germline as well as somatic mutations, indicating the importance of assessing tumor tissue rather than relying on a blood test that assesses germline mutation status only. Clinical activity was also observed in patients without a tBRCA mutation. In these

patients, the nbHRD group (patients whose tumors had a high level of genomic LOH) (n=25) had ORRs of 32% (RECIST) and 40% (RECIST and/or GCIG CA-125), while the biomarker negative group (patients whose tumors had a low level of genomic LOH) (n=13) had a best ORR (RECIST/RECIST & GCIG CA-125) of 8%. The disease control rates across the 3 HRD subgroups displayed very similar differential results. While these preliminary results are very encouraging and indicate that the current analysis approach of assessing BRCA mutation status in tumor tissue and also assessing level of genomic LOH in tumors without a BRCA mutation does differentially identify patients likely respond to rucaparib, the individual group sizes are still small and more data is still required to complete the analysis and determine the optimal HRD signature, particulary with regards to the $BRCA^{wt}$ groups.

Table 1 Response Rates by HRD Subgroup in Part 1 of Study CO-338-017 (ARIEL2)

Efficacy Parameter	HRD Subgroup		
	$tBRCA^{mut}$	nbHRD ($tBRCA^{wt}$ / high LOH)	Biomarker Negative ($tBRCA^{wt}$ / low LOH)
RECIST ORR, % (n)	61 (14/23)	32 (8/25)	8 (1/13)
RECIST & GCIG CA-125 ORR, % (n)	70 (16/23)	40 (10/25)	8 (1/13)
Disease Control Rate* (CR, PR, or SD>12 wks), % (n)	94 (15/16)	75 (9/12)	50 (3/6)
*Patients with SD who are ongoing with <12 weeks on study are not included in denominator			

SUMMARY

Monotherapy rucaparib has demonstrated clinical activity in ovarian cancer patients with and without a BRCA mutation. Overall, response to rucaparib occurs rapidly, with the majority of patients achieving a PR at the first disease assessment scan (weeks 6-8). Responses have been durable and most responders are continuing to receive treatment with rucaparib.

In addition to the data presented by study, the efficacy of rucaparib in $BRCA^{mut}$ ovarian cancer patients who received ≥3 prior chemotherapy regimens and were treated with 600 mg BID rucaparib has been evaluated. In this group, which included both patients with platinum-sensitive and platinum-resistant disease, ORRs of 47% (RECIST) and 73% (RECIST & GCIG CA-125) have been observed, suggesting that rucaparib may be a suitable treatment alternative in this patient population with advanced disease and limited treatment options.

3.3.2.1.3 Pharmacokinetics

After once daily oral administration of rucaparib for 15 days, steady state C_{max} and AUC_{0-24} generally increased dose proportionally. T_{max} and $t_{1/2}$ were independent of dose. Steady state exposure increased by an average of 89%, consistent with accumulation expected for a

compound exhibiting a $t_{1/2}$ of approximately 17 hours administered once daily. Following BID oral administration of rucaparib for 15 days, steady state C_{max} and AUC_{0-24} generally increased dose proportionally. Moreover, BID dosing delivered a lower C_{max} with a low peak to trough plasma concentration variation. The target trough level of 2 μM was achieved in 100% of patients (n=14) at ≥240 mg BID with low inter-patient variability (<4-fold) within each dose group. Steady state trough levels also exhibited low intra-patient variability (24% CV). No sporadically high exposures were observed. The effect of food on rucaparib PK was evaluated at 40 mg (n=3) and 300 mg (n=6) doses administered once daily. There was no food effect; patients may take rucaparib on an empty stomach or with food.

3.3.2.2 Studies A4991002 and A4991005, and A4991014

Further details of these rucaparib combination studies are provided in the Investigator's Brochure.

3.4 Rationale for Study

Clinical data with PARP inhibitors indicate there is an ovarian cancer patient population beyond just those with *gBRCA* mutations and/or platinum-sensitive disease that may benefit from treatment with a PARP inhibitor. The purpose of this study is to test and optimize a molecular signature of HRD in ovarian cancer that is hypothesized to correlate with response to rucaparib and will enable selection of appropriate ovarian cancer patients for treatment with rucaparib. The HRD signature has been defined based on the presence of a deleterious *BRCA1* or *BRCA2* mutation and/or genomic tumor LOH. This study will test the ability of the signature to discriminate good from poor outcome on rucaparib. It is anticipated that patients with a *BRCA1* or *BRCA2* mutation and those with tumors exhibiting tumor genome LOH will derive the greatest clinical benefit from rucaparib treatment.

After optimization (if needed), this signature will be prospectively applied in the final analysis of the planned Phase 3 pivotal study (CO-338-014), which will evaluate rucaparib as switch maintenance treatment following a response to platinum-based chemotherapy in a similar patient population. This Phase 2 study will also compare archival versus recently collected tumor tissue in order to validate the use of archival tumor tissue for assessment of HRD status in the Phase 3 study.

This 2-part study will enroll patients with relapsed, platinum-sensitive, high-grade epithelial ovarian, fallopian tube, and primary peritoneal cancer who have disease that can be biopsied and is measurable. Part 1 of the study will enroll approximately 180 patients who received ≥1 prior platinum-based regimen and have platinum-sensitive disease. Enrollment of patients known to harbor a deleterious / pathogenic *gBRCA* mutation will be limited to 15 in Part 1 in order to enrich for patients that have HRD associated with a defect other than *BRCA1/2*. Part 2 will enroll up to 300 patients who received at least 3, but no more than 4, prior chemotherapy regimens, including at least 80 patients with a *tBRCA* mutation. Patients will enroll into either Part 1 or Part 2 of the study. Part 2 will begin once enrollment of Part 1 has been completed.

Tumor tissue collected at screening and/or an archival tumor tissue sample will be sequenced using Foundation Medicine's next generation sequencing (NGS) test, which analyzes a large

panel of cancer-related genes, including tumor genome LOH, *BRCA*, and other HRR pathway genes. Genetic alterations, which include mutations and homozygous deletions, in specific HRR pathway genes may also be associated with clinical outcome on treatment with rucaparib.

The following correlative translational studies are also planned:

1. Tumor genomic LOH and gene sequence alterations in archival and screening tumor tissue will be compared to assess the changes in a tumor's genomic LOH and genetic profile over time and determine if archival tumor tissue carries sufficient predictive utility and obviates the need for a contemporaneous biopsy. The frequency and nature of secondary *BRCA* mutations will also be assessed. Acquired secondary *BRCA* mutations (also known as reversions) may result in functional protein and restored HRR capability, leading to PARP inhibitor resistance.[16-18]
2. An alternative NGS test known as BROCA will be used to potentially identify additional mutations in other DNA repair genes that may confer sensitivity or resistance to rucaparib.[19]
3. Gene expression profiling on extracted RNA will be analyzed to potentially identify a signature associated with efficacy. A gene expression signature has been developed to identify *BRCA* and *BRCA*-like (also referred to as "BRCAness") tumors. Such a signature may predict response to platinum and PARP inhibitors.[20]
4. Immunohistochemistry (IHC) of non-homologous end joining (NHEJ) proteins will be investigated to assess whether NHEJ pathway integrity modulates efficacy. It has been hypothesized that cells with HRD must have functional NHEJ DNA repair in order to generate sufficient genomic instability for synthetic lethality with a PARP inhibitor.[21]
5. Circulating cell-free tumor DNA (ctDNA) will be analyzed as a potential molecular marker of efficacy. Tagged-amplicon deep sequencing (TAm-Seq) will be utilized to sequence ctDNA and identify mutations, including but not limited to, those in the tumor suppressor gene TP53, which is present in greater than 95% of HGSOC tumors.[1,22] Similar to CA-125, the fraction of TP53 mutant alleles in plasma of ovarian cancer patients has been shown to track with the clinical course of the disease.[22]

Taken together, the analyses planned in this trial will provide valuable information on genomic abnormalities that may be associated with response or resistance to rucaparib and will identify a broader ovarian cancer patient population that may benefit from treatment than has previously been explored in most other PARP inhibitor studies.

4 STUDY OBJECTIVES

4.1 Objectives and Endpoints

This is a two-part, open-label efficacy study of oral rucaparib in patients with platinum-sensitive, relapsed, high-grade epithelial ovarian, fallopian tube, or primary peritoneal cancer to define a signature of HRD that correlates with efficacy.

Primary, secondary, and exploratory objectives and endpoints are shown in Table 2. Unless otherwise specified, the objectives below apply to both parts of the study.

Table 2 Primary, Secondary, and Exploratory Objectives and Endpoints

Primary Objectives	**Primary Endpoints**
1. To determine PFS in patients with relapsed platinum-sensitive ovarian cancer classified into molecularly-defined subgroups by a prospectively defined HRD signature (Part 1)	1. Disease progression (RECIST v1.1) (Appendix B) as assessed by investigator, or death from any cause, in molecularly-defined subgroups identified by a prospectively defined HRD signature
2. To estimate ORR in heavily pre-treated patients with relapsed ovarian cancer classified into molecularly-defined subgroups by a prospectively defined HRD signature (Part 2)	2. ORR by RECIST v1.1 in molecularly-defined subgroups identified by a prospectively defined HRD signature
Secondary Objectives	**Secondary Endpoints**
1. To estimate ORR (Part 1)	1. ORR by RECIST v1.1
2. To estimate ORR including CA-125 response criteria	2. ORR by RECIST v1.1 and GCIG CA-125 criteria
3. To evaluate duration of response (DOR)	3. DOR by RECIST v1.1
4. To determine PFS (Part 2)	4. Disease progression (RECIST v1.1) (Appendix B) as assessed by investigator, or death from any cause
5. To evaluate survival (Part 2)	5. Overall survival
6. To evaluate the safety and tolerability of rucaparib	6. The incidence of adverse events (AEs), clinical laboratory abnormalities, and dose modifications
7. To evaluate steady state trough level PK	7. Trough (C_{min}) level rucaparib concentrations

Table 2 Primary, Secondary, and Exploratory Objectives and Endpoints	
Exploratory Objectives	**Exploratory Endpoints**
1. To assess efficacy in molecularly-defined HRD subgroups as defined by HRR gene alterations	1. PFS and/or ORR by RECIST v1.1 and GCIG CA-125 criteria. HRD subgroups as defined by HRR gene alterations
2. To optimize the tumor LOH algorithm by testing additional signatures of interest based on higher or lower genomic LOH	2. PFS and/or ORR by RECIST v1.1 and GCIG CA-125 criteria. Additional signatures of interest based on higher or lower genomic LOH.
3. To assess changes in HRD status over time	3. Changes in HRD status (LOH and gene alterations) between fresh biopsy versus archival tumor tissue samples
4. To assess whether the BROCA panel can identify mutations in additional HRR genes that may be associated with efficacy	4. ORR by RECIST v1.1 and GCIG CA-125 criteria in relation to HRR gene mutations identified in BROCA
5. To assess if a gene expression signature for HRD correlates with efficacy	5. PFS and/or ORR by RECIST v1.1 and GCIG CA-125 criteria in relation to gene signature defined by a gene expression profiling assay
6. To assess NHEJ pathway integrity and correlate it with efficacy	6. NHEJ protein expression by immunohistochemistry (IHC) and PFS and/or ORR by RECIST v1.1 and GCIG CA-125 criteria
7. To assess ctDNA as a molecular marker of efficacy	7. Levels of ctDNA in relation to PFS and/or ORR by RECIST v1.1 and GCIG CA-125 criteria

5 STUDY DESIGN

5.1 Overall Study Design and Plan

This is a two-part, open-label efficacy study of rucaparib in patients with platinum-sensitive, relapsed, high-grade epithelial ovarian, fallopian tube, or primary peritoneal cancer to characterize the relationship between HRD status and rucaparib efficacy in patients who received ≥1 prior platinum-based regimen and have platinum-sensitive disease (Part 1) and patients who received at least 3, but no more than 4, prior chemotherapy regimens (Part 2). Patients will enroll into either Part 1 or Part 2 of the study. Part 2 will begin once enrollment of Part 1 has been completed.

5.1.1 Screening Phase

All patients will undergo screening assessments within 28 days prior to the first dose of rucaparib. AEs that occur after signing of the informed consent form and before administration of the first rucaparib dose will also be collected during this period.

Screening assessments will include demographics and medical history, prior treatments for epithelial ovarian, fallopian tube, or primary peritoneal cancer (and other malignancies if applicable), prior and current medications, and procedures, 12-lead electrocardiogram (ECG), ECOG performance status, hematology, serum chemistry, serum pregnancy for women of childbearing potential, urinalysis, blood sample for ctDNA analysis, physical examination, vital signs, weight and height measurements, adverse events, radiological assessment by CT or MRI, and CA-125 measurement. All patients, with the exception of Part 2 patients known to harbor a deleterious *gBRCA* mutation, will be required to have a screening biopsy to collect fresh tumor tissue for determination of HRD status at study entry within 28 days prior to the first dose of rucaparib. The screening biopsy will be optional for Part 2 patients known to harbor a *gBRCA* mutation. This biopsy should be performed at least 7 days prior to the planned start of treatment to allow sufficient time for the sample to be sent to Foundation Medicine, the central laboratory for confirmation the tissue is of adequate quality for the planned analyses. If a biopsy was recently performed as standard of care prior to this patient consenting to this study or after study informed consent but outside the 28 day screening window this may be acceptable with advance approval from the Sponsor. In addition, archival tumor tissue samples must be confirmed as being available for all patients. While archival tumor tissue is not required to be shipped prior to initiation of treatment, it is highly recommended that the tissue be sent as close as possible to the time of sending the screening biopsy to enable timely enrollment in the event that the screening biopsy is deemed inadequate by Foundation Medicine.

In Part 1, patients <55 years of age at diagnosis, or with prior history of breast cancer, or who have a close relative (first or second degree) with ovarian cancer or early onset (<age 50) breast cancer are required to have been previously tested for *gBRCA* mutation. Germline *BRCA* test results must be obtained for all patients who are known to have been tested prior to enrollment in order to determine whether any mutation was reported and if so, whether the mutation was classified as deleterious / pathogenic or other. Enrollment of patients with a *gBRCA* mutation classified as deleterious (i.e., pathogenic), suspected deleterious, or favor deleterious (or the equivalent interpretation of any of these) on the most recent assessment by a testing laboratory

will be limited to 15 in Part 1. Patients not required to have been previously tested, or who tested negative for a *gBRCA* mutation, or who were found to have a mutation that was classified as other than deleterious, suspected deleterious, or favor deleterious or the equivalent of any of these, are eligible to enroll in Part 1 provided all other criteria are met. Patients with a *BRCA* mutation detected in tumor tissue (*tBRCA*), but who do not have a germline mutation, will not count toward the cap and will be eligible to receive treatment with rucaparib, provided all other eligibility criteria are met.

At least 80 patients with a deleterious / pathogenic *tBRCA* mutation will be enrolled into Part 2 of the study. There is no requirement for a patient enrolling into Part 2 to have been previously tested for a germline *BRCA* mutation, even if she meets the clinical criteria for testing being applied to patients entering Part 1 of the study.

Results of the Foundation Medicine panel test will be provided to all patients who consent to receive this information. In the event a *BRCA1* or *BRCA2* mutation is detected in tumor tissue, the patient may be referred by the investigator for genetic counseling and potential germline testing per institutional guidelines. If the patient chooses to have germline testing, this result will be entered into the clinical trial database.

Mutations detected in tumor tissue may be somatic or germline; however, the central laboratory's NGS test will not distinguish between the two. A blood sample will therefore be collected for all patients at screening and stored. Prior to final efficacy analysis, genomic DNA may be subjected to exploratory analysis in order to determine whether the mutation is germline. These data will be generated in a research setting and will not be provided to the investigator or patient.

Enrollment will require Clovis review of eligibility, including information on prior cancer therapies and dates administered, local *gBRCA* test result if patient has previously been tested, and, with the exception of Part 2 patients known to harbor a deleterious *gBRCA* mutation, confirmation that the screening biopsy sample has been submitted to the central laboratory and deemed adequate for the planned genetic analyses. Confirmation that an adequate amount of archival tumor tissue is available for analysis is also required.

5.1.2 Treatment Phase

During the treatment phase (continuous 28-day treatment cycles), patients will be monitored for safety and efficacy. Assessments during the treatment phase will include AEs, ECOG performance status, concomitant medications and procedures, physical examination, vital signs and weight measurements, hematology and serum chemistry, alpha-1 acid glycoprotein (AAG) analysis on days where a blood sample is taken for PK, serum or urine pregnancy (per investigator discretion) for women of childbearing potential, CA-125 measurement, blood samples for PK and ctDNA, and study drug administration and accountability. Patients will be assessed for disease status per RECIST v1.1 at the end of every 8 weeks (± 4 days) during the treatment phase. Confirmatory scans should be performed at least 4 weeks after an initial PR or CR is first documented. Patients who have been on study at least 18 months may decrease the frequency of disease assessments to every 16 (±2) weeks. Patients experiencing disease progression, as assessed by the investigator, will be discontinued from treatment.

A formal safety data review will occur after the first 20 patients have been enrolled, then every quarter until Part 1 enrollment is completed, and then every 6 months thereafter. The review committee will include external experts and Sponsor personnel. The external experts will include, but not be limited to, the coordinating PIs of the study (Dr. Elizabeth Swisher at Univ. of Washington and Dr. Iain McNeish at Univ. of Glasgow). Clovis reviewers will include the Medical Monitor, Chief Medical Officer, Head of Pharmacovigilance, and Biostatistician. The protocol will be amended as appropriate to incorporate additional patient safety monitoring if new safety signals are noted at any review.

5.1.3 Post-Treatment Phase

Upon discontinuation of treatment with rucaparib, all patients will return to the clinic for an End of Treatment visit. Assessments at this visit will include AEs, ECOG performance status, concomitant medications and procedures, 12-lead ECG, physical examination, vital signs and weight measurements, hematology and serum chemistry, serum pregnancy for women of childbearing potential, CA-125 measurement, blood sample for ctDNA analysis, disease status assessment, and study drug accountability. An optional tumor biopsy sample, if available, will be collected from patients who experience disease progression and provide appropriate consent. Disease assessments should also be done at the time of treatment discontinuation if it has been ≥ 8 weeks since the last assessment.

All patients will be assessed for AEs at 28 (±3) days following the last dose of rucaparib at the 28-Day Follow-up visit.

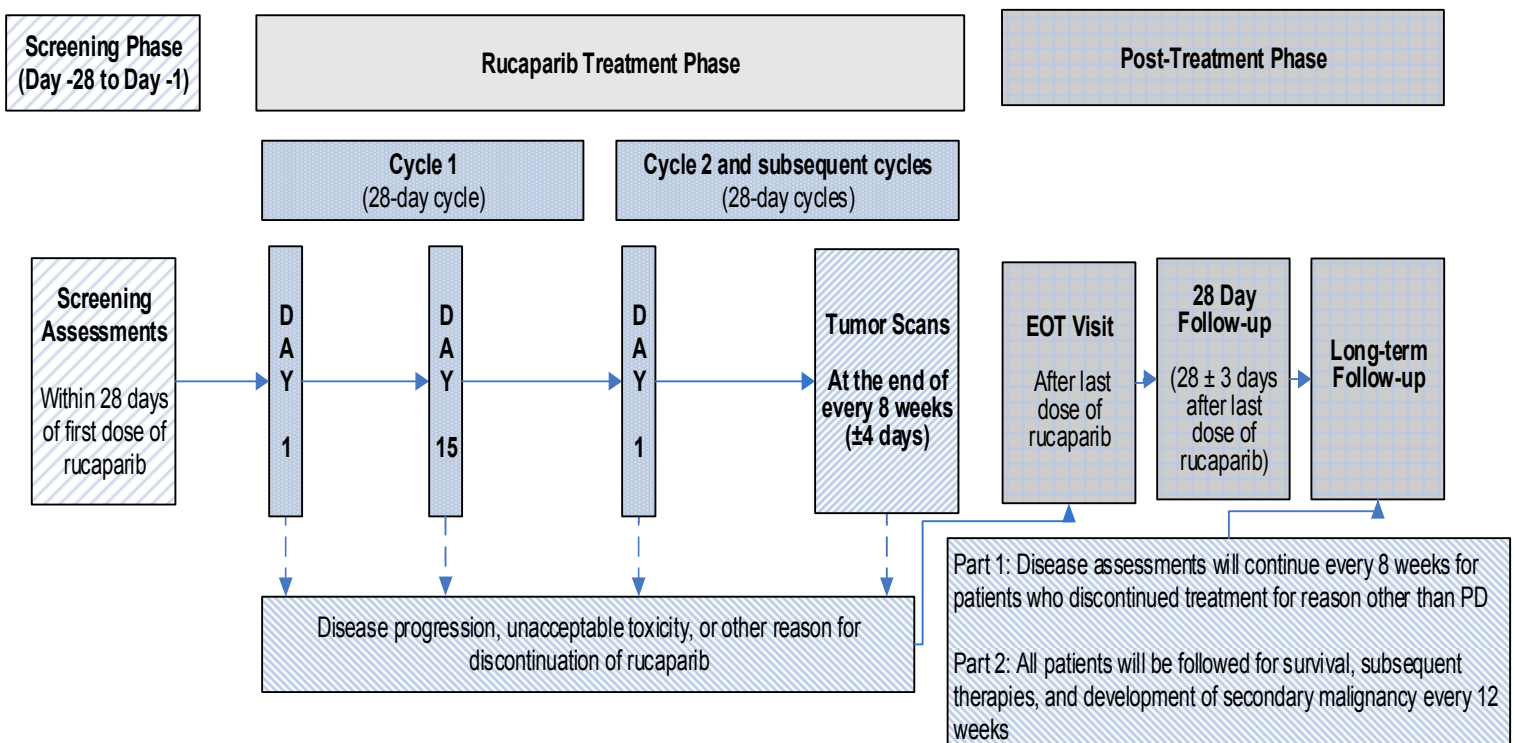
All patients that discontinued treatment for any reason other than radiogically confirmed disease progression will continue to have scans every 8 weeks (± 4 days) until radiologically confirmed disease progression, death or initiation of subsequent treatment. Patients who have been on study at least 18 months, may decrease the frequency of disease assessments to every 16 (±2) weeks.

Patients in Part 2 of the study will also be followed for survival, subsequent treatments, and monitoring for secondary malignancy every 12 weeks until death, loss to follow-up, withdrawal of consent, or study closure.

5.2 Study Schema

The study schema in Figure 3 summarizes the treatment design of the study.

Figure 3 Study Schema



5.3 End of Study

The trial will close when all patients in Part 2 have experienced death or have been followed for survival for a period of 2 years, whichever occurs first. Upon formal closure of the study, individual patients who are continuing to receive benefit from treatment at the time of study closure, and who do not meet any of the criteria for withdrawal, will have the option of entering an extension protocol in which they can continue to receive rucaparib.

6 STUDY POPULATION

6.1 Number of Patients and Sites

Approximately 480 patients with platinum-sensitive, relapsed high-grade epithelial ovarian, fallopian tube, or primary peritoneal cancer will be enrolled into either Part 1 (n=180) or Part 2 (n=300) of the study at approximately 60 study sites. Enrollment of patients known a priori to have a deleterious / pathogenic *gBRCA* mutation will be limited to 15 in Part 1. At least 80 patients with a *tBRCA* mutation will be enrolled into Part 2.

6.2 Inclusion Criteria

Eligible patients must meet the following inclusion criteria. Unless otherwise specified, the criteria below apply to patients enrolling in either Part 1 or Part 2 of the study.

1. Have signed an Institutional Review Board (IRB)/Independent Ethics Committee (IEC)-approved informed consent form prior to any study-specific evaluation
2. Be ≥18 years of age at the time the informed consent form is signed
3. Have a histologically confirmed diagnosis of high-grade serous or Grade 2 or Grade 3 endometrioid epithelial ovarian, fallopian tube, or primary peritoneal cancer
   - If mixed histology, >50% of the primary tumor must be confirmed to be high-grade serous or endometrioid upon re-review by local pathology
   - Patients with a histology other than serous or endometrioid are also eligible for Part 2 of the study if they are known to harbor a deleterious / pathogenic *BRCA* mutation (germline or somatic)
4. Have relapsed/progressive disease as confirmed by radiologic assessment
5. **Part 1**: Received prior platinum-based therapy and have platinum-sensitive disease
   a. Received ≥1 prior platinum-based treatment regimen; AND
   b. Received a platinum-based regimen as their last treatment; continuous or switch maintenance treatment as part of this regimen is permitted (hormonal treatment may be permitted following the last platinum regimen with advance approval from the Sponsor); AND
   c. Was sensitive to the last platinum regimen. Platinum-sensitive disease is defined as documented radiologic progression ≥6 months after the last dose of platinum administered in the treatment setting.

   Part 2: Received at least 3, but no more than 4, prior chemotherapy regimens and had documented treatment-free interval of ≥6 months following 1st chemotherapy regimen received
   a. Hormonal agents (eg. tamoxifen, letrozole, etc), anti-angiogenic agents (eg. bevacizumab, pazopanib, cediranib, nintedanib, trebananib, etc), and other non-chemotherapy agents administered as single agent treatement will not be counted as a chemotherapy regimen for the purpose of determing patient eligibility
   b. Agents administered in the maintenance setting will not be counted as a separate regimen

6. **Part 1 only:** If <55 years of age at diagnosis, or has prior history of breast cancer, or has close relative (first or second degree) with ovarian cancer or early onset (<age 50) breast cancer, must have been previously tested for *gBRCA* mutation ; after 15 patients harboring the *gBRCA* mutation are enrolled, no additional patients with a known *gBRCA* mutation will be allowed to enroll.
7. Have undergone a biopsy of tumor tissue prior to first dose of study drug and had the tumor tissue confirmed by the central laboratory as being of adequate quality (at least 20% tumor content with a minimum of 80% nucleated cellular content). *Note: biopsy is optional for Part 2 patients known to harbor a deleterious gBRCA mutation*
   - If tumor tissue obtained from the biopsy is deemed inadequate, and the patient is unwilling or unable to have another biopsy, the patient may be considered for enrollment if archival tumor tissue is provided and deemed of adequate quality. This must occur prior to any treatment with rucaparib.
     a. Biopsy must be of solid tumor tissue; ascites is not acceptable.
     b. Biopsy must be of sufficient yield for planned analyses
8. Have sufficient archival FFPE tumor tissue available for planned analyses; cytospin blocks from ascites are not acceptable
   - The most recently obtained tumor tissue that is of adequate quality (at least 20% tumor content with a minimum of 80% nucleated cellular content) should be submitted
9. Have measurable disease as defined by RECIST v1.1 (Appendix B) in addition to the lesion planned for biopsy; a single RECIST target lesion will suffice if, in the Investigator's opinion, it is of sufficient size that the biopsy will not affect post-dose RECIST evaluations.
10. Have adequate organ function confirmed by the following laboratory values obtained within 14 days prior to the first dose of rucaparib:
    a. Bone Marrow Function
       i. Absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/L
       ii. Platelets $>100 \times 10^9$/L
       iii. Hemoglobin ≥9 g/dL
    b. Hepatic Function
       i. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤3 × upper limit of normal (ULN); if liver metastases, then ≤5 × ULN
       ii. Bilirubin ≤1.5 × ULN (<2 × ULN if hyperbilirubemia is due to Gilbert's syndrome)
       iii. Serum albumin ≥30 g/L (3 g/dL) (Part 2 only)
    c. Renal Function
       i. Serum creatinine ≤1.5 × ULN or estimated glomerular filtration rate (GFR) ≥45 mL/min using the Cockcroft Gault formula
11. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1 (Appendix C)

6.3 Exclusion Criteria

Patients will be excluded from participation if any of the following criteria apply. Unless otherwise specified, the criteria below apply to patients enrolling in either Part 1 or Part 2 of the study.

1. Active second malignancy, i.e., patient known to have potentially fatal cancer present for which she may be (but not necessarily) currently receiving treatment
    a. Patients with a history of malignancy that has been completely treated, with no evidence of that cancer currently, are permitted to enroll in the trial provided all chemotherapy was completed >6 months prior and/or bone marrow transplant (BMT) >2 years prior to first dose of rucaparib
2. Prior treatment with any PARP inhibitor, including oral or intravenous rucaparib. Patients who previously received iniparib are eligible
3. Symptomatic and/or untreated central nervous system (CNS) metastases. Patients with asymptomatic previously treated CNS metastases are eligible provided they have been clinically stable for at least 4 weeks
4. Pre-existing duodenal stent and/or any gastrointestinal disorder or defect that would, in the opinion of the Investigator, interfere with absorption of rucaparib
5. Known human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS)-related illness, or history of chronic hepatitis B or C
6. Pregnant or breast feeding. Women of childbearing potential must have a negative serum pregnancy test <3 days prior to first dose of rucaparib
7. Received treatment with chemotherapy, radiation, antibody therapy or other immunotherapy, gene therapy, vaccine therapy, angiogenesis inhibitors, or experimental drugs ≤14 days prior to first dose of rucaparib and/or ongoing adverse effects from such treatment > NCI CTCAE Grade 1 (ongoing Grade 2 non-hematologic toxicity related to most recent treatment regimen may be permitted with prior advanced approval from Sponsor).
8. Received administration of strong CYP1A2 or CYP3A4 inhibitors ≤7 days prior to first dose of rucaparib or have on-going requirements for these medications (Appendix D)
9. Non-study related minor surgical procedure ≤5 days, or major surgical procedure ≤21 days, prior to first dose of rucaparib; in all cases, the patient must be sufficiently recovered and stable before treatment administration
10. Presence of any other condition that may increase the risk associated with study participation or may interfere with the interpretation of study results, and, in the opinion of the investigator, would make the patient inappropriate for entry into the study
11. Diagnosis of low-grade serous or Grade 1 endometrioid ovarian cancer
12. **Part 2 Only:** Hospitalization for bowel obstruction within 3 months prior to enrollment

6.4 Patients or Partners of Patients of Reproductive Potential

Pregnancy is an exclusion criterion and women of childbearing potential must not be considering getting pregnant during the study. Female patients who are more than 2 years postmenopausal or have had a hysterectomy and/or bilateral oophorectomy will not be considered of childbearing potential. Female patients of childbearing potential must have a negative serum pregnancy test result less than 3 days prior to administration of the first dose of rucaparib. In addition, a serum or urine pregnancy test (per investigator discretion) must be performed <3 days prior to Day 1 of every cycle during the treatment phase. A serum pregnancy test will be performed at the End of Treatment visit.

Female patients of reproductive potential and their male partners must practice an effective method of contraception during treatment and for 6 months following the last dose of rucaparib. Adequate contraception is defined as double-barrier method (i.e., condom in combination with a diaphragm, cervical/vault cap, or intrauterine device). Oral, injectable, implant, or patch forms of contraception are not permitted as potential drug-drug interaction between rucaparib and these forms of birth control has not yet been evaluated.

Patients will be instructed to notify the investigator if pregnancy is discovered either during or within 6 months of completing treatment with rucaparib.

6.5 Waivers of Inclusion/Exclusion Criteria

No waivers of these inclusion or exclusion criteria will be granted by the investigator and the sponsor or its designee for any patient enrolling into the study.

7 DESCRIPTION OF STUDY TREATMENTS AND DOSE MODIFICATIONS

7.1 Description of Investigational Product

Rucaparib camsylate (formerly known as PF-01367338 and AG-014447) is an oral formulation with a molecular weight of 555.67 Daltons. Rucaparib tablets for oral administration will be supplied to the study sites by the sponsor. A brief description of the investigational product is provided below.

Drug Name:	CO-338
INN:	Rucaparib
Manufacturer:	Drug substance: Lonza Limited Chemie, Visp, Switzerland Drug product: Pfizer Manufacturing Deutschland GmbH, Freiburg, Germany
Formulation:	Tablet; film coated; 60 mg – white, 120 mg – salmon, 200 mg – blue, 300 mg – yellow
How Supplied:	60, 120, 200 and/or 300 mg (as free base) strength in high-density polyethylene bottles or equivalent with child-resistant caps. Patients may receive one or more strengths.
Storage Conditions:	15–30 °C

Study drug containers containing rucaparib tablets will be labeled according to national regulations for investigational products. Where accepted, the expiry date will not appear on the labels, but will be controlled by the use of an Interactive Voice Response System/Interactive Web Response System (IVRS/IWRS).

7.2 Method of Assigning Patients to Treatment Groups

All patients enrolled in the study will receive rucaparib.

7.3 Preparation and Administration of Protocol-Specified Treatment

The investigator or designee will be responsible for distributing rucaparib tablets to all patients. Study sites should follow local guidelines for the handling of oral cytotoxic drugs.

All patients will ingest rucaparib twice a day. Patients may take rucaparib on an empty stomach or with food. Each dose should be taken with at least 8 oz (240 mL) of room temperature water. Tablets should be swallowed whole.

Patients should take rucaparib doses as close to 12 hours apart as possible, preferably at the same times every day. If a patient misses a dose (i.e., does not take it within 4 hours of the scheduled time), she should skip the missed dose and resume taking rucaparib with her next scheduled dose. Missed or vomited doses should not be made up.

A sufficient number of tablets will be provided to the patient to last until the next scheduled visit. Patients will be instructed to record daily doses taken or not taken on a patient diary, and will be

instructed to bring their rucaparib tablets and all containers (empty, partially used, and/or unopened) and diary to the next scheduled visit for reconciliation by site personnel.

Patients enrolled into Part 1 of the study will initially receive 120 mg tablets. Patients enrolled into Part 2 of the study will initially receive 300 mg tablets. (Tablets of 200 mg dose strength will also be available for patients in Part 2 to enable dose reductions in 100 mg increments – see Table 3).

- Once available supplies of 120 mg tablets are exhausted, patients receiving this dose strength will be transitioned to 300 mg tablets.
- Transition of patients from dosing with 300 to 120 mg tablets is unlikely, but will be implemented if necessary based on availability of clinical supplies or other reason
- The dose that a patient will receive upon transition from 120 to 300 mg tablets (or 300 to 120 mg tablets if needed) will be agreed upon between the Investigator and Sponsor (or designee) in advance of any dosing change

7.3.1 Dietary Restrictions

All patients participating in the study should be instructed not to eat or drink any grapefruit products or any of the CYP1A2 and CYP3A4 inhibitors noted in Appendix D for 7 days prior to their first dose of rucaparib and for the duration of their participation on the study. In addition, once patients begin taking rucaparib, they should be instructed not to consume products containing star fruit, Seville orange, pomegranate, pummelo or their juices.

7.4 Starting Dose and Dose Modifications of Protocol-Specified Treatment

7.4.1 Starting Dose

The starting dose in this study will be 600 mg rucaparib BID. This dose was selected as the recommended dose for future Phase 2 and Phase 3 studies based on safety, tolerability, overall PK, and preliminary efficacy profile observed in the CO-338-010 study, which evaluated monotherapy rucaparib in patients with advanced solid tumors. A summary of that study is provided in Section 3.3.2.1. In the event that the recommended Phase 2 dose of 600 mg BID rucaparib is determined to be unsuitable for chronic dosing, the starting dose may be decreased to Dose Level -1 (480 mg BID rucaparib) for all subsequent patients if agreed upon between the Sponsor and the Principal Investigators.

7.4.2 Dose Modification Criteria

Treatment with rucaparib should be held if any of the following are observed and a dose reduction should be considered or implemented.

- Grade 3 or 4 hematologic toxicity

- Grade 3 or 4 non-hematologic toxicity (except for alopecia, nausea, vomiting, or diarrhea adequately controlled with systemic antiemetic/antidiarrheal medication administered in standard doses according to the study center routines)
  - Note: rucaparib is not required to be held for Grade 3 elevations of ALT/AST if not accompanied by other signs of liver dysfunction
- In addition, and at the discretion of the investigator, the dose of rucaparib may be held and/or reduced for Grade 2 toxicity not adequately controlled by concomitant medications and/or supportive care.

Treatment with rucaparib should be held until the toxicity resolves to ≤CTCAE Grade 2. Twice daily dosing may then be resumed at either the same dose or a lower dose, per investigator discretion. If treatment is resumed at the same dose, and the patient experiences the same toxicity, the dose should be reduced following resolution of the event to ≤CTCAE Grade 2. If the patient continues to experience toxicity, additional dose reduction steps are permitted. If a patient continues to experience toxicity despite multiple dose reduction steps, or if dosing with rucaparib is interrupted for >14 consecutive days due to toxicity, treatment should be discontinued, unless otherwise agreed between the investigator and the sponsor.

Dose reduction steps are presented in Table 3.

Dose re-escalation upon resolution of toxicity to ≤CTCAE Grade 1 is permitted upon agreement between the investigator and Sponsor.

Table 3 Dose Reduction Steps

Tablets	**60/120 mg**	**300/200 mg**
Starting Dose	**600 mg BID**	
Dose Level -1	480 mg BID	500 mg BID
Dose Level -2	360 mg BID	400 mg BID
Dose Level -3*	240 mg BID	300 mg BID
*Consult with medical monitor before reducing to this dose		

7.4.3 Criteria for Re-Treatment

A new cycle of treatment may begin if:

- ANC ≥1.0 x 109/L
- Platelet count ≥100 x 109/L

- Non-hematologic toxicities have returned to baseline or ≤CTCAE Grade 1 severity (or, at the investigator's discretion, ≤CTCAE Grade 2 severity if not considered a safety risk for the patient)

7.4.4 Treatment Beyond Progression

If the patient has met criteria for radiologic progression by RECIST, but the patient is still receiving benefit from rucaparib (e.g., patient has mixed radiologic response or is continuing to have symptomatic benefit) according to the Investigator, then continuation of treatment will be considered. In such cases, the decision to continue will be made jointly between the Investigator and the Sponsor, and must be documented prior to continuing treatment with rucaparib. Patients will continue to have all protocol-required assessments specified in Table 4.

7.5 Accountability of Protocol-Specified Treatment

Study personnel will maintain accurate records of study drug receipt, dispensation, use, return, destruction, and reconciliation. A web/phone-based drug management system will be used to manage study drug inventory at all sites. In order to function properly, the system will require real-time entry of study drug receipt, dispensation, destruction, etc. by study personnel at the study center.

The site is responsible for the return or destruction of study drug as required. Any study drug accidentally or deliberately destroyed must be accounted for. All study drug containers must be accounted for prior to their destruction at the study center, according to institutional procedures for disposal of cytotoxic drugs. Unused study drug containers should be destroyed on-site if possible. If destruction on site is not possible, supply should be returned to the drug depot.

During the course of the study and at completion of the study, the number of study drug containers received, dispensed, returned, and destroyed must be reconciled.

7.6 Blinding/Masking of Treatment

This is an open-label study; the investigational product will not be blinded or masked. All patients enrolled will receive rucaparib.

7.7 Treatment Compliance

Documentation of dosing will be recorded in a study specific drug dosing diary provided by the sponsor (or designee). Study site personnel will review dosing information with the patient (or legally authorized representative) on scheduled clinic visit days, providing instructions regarding dose, dose frequency and the number of tablets to be taken for each dose. Patients (or legally authorized representative) will be instructed to record dosing information for rucaparib taken at home in the drug dosing diary and to bring the drug dosing diary and all unused tablets with them to scheduled clinic visits. A compliance check and tablet count will be performed by study personnel during clinic visits. Study site personnel will record compliance information on the electronic case report form (eCRF) and retain the drug dosing diary in the patient's medical record.

Every effort should be made to ensure patients complete the dosing diary in full and return it to the clinic along with their study drug containers at the end of each cycle of treatment. In the event a patient does not return their dosing diary, study site personnel should conduct a verbal review of dosing with the patient and document the discussion in the patient's medical record. This may serve as source documentation for the purpose of entering dosing data on the appropriate eCRF.

8 PRIOR AND CONCOMITANT THERAPIES

Patients who have received prior treatment with a PARP inhibitor including intravenous or oral rucaparib are not eligible to participate in this study. Patients having received prior treatment with iniparib are eligible.

During the study, supportive care (e.g., antiemetics; analgesics for pain control) may be used at the investigator's discretion and in accordance with institutional procedures.

All procedures performed (e.g., thoracentesis, etc.) and medications used during the study must be documented on the eCRF.

8.1 Anticancer or Experimental Therapy

No other anticancer therapies (including chemotherapy, radiation, antibody or other immunotherapy, gene therapy, vaccine therapy, angiogenesis inhibitors, or other experimental drugs) of any kind will be permitted while the patient is participating in the study with the exception of hormonal treatment. Prior treatment with such excluded anticancer therapies must have been completed >14 days prior to the first dose of study drug.

8.2 Hematopoietic Growth Factors and Blood Products

Erythropoietin, darbepoetin alfa, and/or hematopoietic colony-stimulating factors for treatment of cytopenias should be administered according to institutional guidelines. Transfusion thresholds for blood product support will be in accordance with institutional guidelines.

8.3 CYP450 Isoenzyme Inhibitors, Inducers, and Substrates

The plasma concentrations of rucaparib may be increased in the presence of co-administered potent CYP1A2 or CYP3A4 inhibitors. Therefore, strong CYP1A2 and CYP3A4 inhibitors are excluded. Moderate inhibitors are permitted at the discretion of the Investigator in the event a suitable alternative cannot be found.

The plasma concentrations of rucaparib may be reduced in the presence of co-administered potent CYP1A2 or CYP3A4 inducers. Therefore, strong CYP1A2 and CYP3A4 inducers are excluded. Moderate inducers are permitted at the discretion of the Investigator in the event a suitable alternative cannot be found. In addition, CYP1A2 is known to be induced in chronic smokers. Smokers are not excluded from the study; however, smoking status should be assessed and recorded in the source documents and eCRF.

A list of CYP1A2 and CYP3A4 inhibitors and inducers to be avoided or used with caution is provided in Appendix D.

Because rucaparib was shown to be a moderate inhibitor of CYP1A2, CYP2C8, CYP2C9, and CYP2C19 in vitro, caution should also be exercised in patients receiving rucaparib and requiring concomitant medication with CYP substrates that have a narrow therapeutic range, such as paclitaxel, phenytoin, *S*-mephenytoin, theophylline, tizanidine, and warfarin (Coumadin), as

rucaparib doses ≥480 mg might increase the plasma concentration of these medications. Concomitant administration of paclitaxel is not permitted in this study. Other susceptible medications should be used with caution with monitoring of plasma levels and/or pharmacodynamic surrogates as appropriate.

8.4 Bisphosphonates

Bisphosphonates are permitted.

8.5 Anticoagulants

Caution should be exercised in patients receiving rucaparib and concomitant warfarin (Coumadin) as rucaparib showed a mixed inhibition of CYP2C9 in vitro. If appropriate, low molecular weight heparin should be considered as an alternative treatment. Patients taking warfarin should have INR monitored regularly per standard clinical practice.

8.6 Other Concomitant Medications

Therapies considered necessary for the patient's well-being may be given at the discretion of the investigator and should be documented on the eCRF. Other concomitant medications, except for analgesics, chronic treatments for concomitant medical conditions, or agents required for life-threatening medical problems, should be avoided. Herbal and complementary therapies should not be encouraged because of unknown side effects and potential drug interactions, but any taken by the patient should be documented appropriately on the eCRF.

In vitro data showed that rucaparib is an inhibitor of P-gp and thus patients taking digoxin, a P-gp substrate, should have their digoxin levels monitored regularly via standard clinical practice.

9 STUDY PROCEDURES

9.1 Schedule of Assessments

Table 4 summarizes the procedures and assessments to be performed for all patients.

All procedures and assessments are to be completed within ±3 days of the scheduled time point.

Table 4 Schedule of Assessments

Procedure[a]	Screening Phase	Treatment Phase (±3 days of scheduled timepoint)			Post-Treatment Phase		
		Cycle 1		Cycles 2+			
	Day -28 to Day -1	Day 1[b]	Day 15	Day 1	End of Treatment	28 Day Follow-up (FU) (28 ± 3 days after last dose)	Long-term FU
Informed Consent[c]	X						
Medical/Oncology History[d]	X						
Physical Examination[e], Height[e], Weight	X	X		X	X		
ECOG Performance Status	X	X		X	X		
Vital Signs	X	X[f]		X[f]	X		
Adverse Events[g]	X	X	X	X	X	X	
Prior/Concomitant Medications and Procedures	X	X	X	X	X		
12-lead ECG[h]	X				X		
Hematology[i] (local lab)	X[j]	X	X	X	X		
Serum Chemistry[k] (local lab)	X[j]	X	X	X	X		
Pregnancy Test[l] (WOCBP only) (local lab)	X			X	X		
Urinalysis[m] (local lab)	X						
Disease Assessment/Tumor Scans[n]	X			X[o]	(X)[p]		X[q]
CA-125 Measurement	X[r]	X		X[s]	X		
Tumor Tissue Biopsy	X[t]				(X)[u]		
Archival Tumor Tissue	X[v]						

Table 4 Schedule of Assessments

Procedure[a]	Screening Phase	Treatment Phase (±3 days of scheduled timepoint)			Post-Treatment Phase		
		Cycle 1		Cycles 2+			
	Day -28 to Day -1	Day 1[b]	Day 15	Day 1	End of Treatment	28 Day Follow-up (FU) (28 ± 3 days after last dose)	Long-term FU
Blood Sample for ctDNA Analysis[w] (central lab)	X	X		X	X		
Blood Sample for Storage (central lab)	X						
Rucaparib Dispensation/Administration/ Accountability		X		X	X		
Plasma PK Sample[x] (central lab)			X	X[y]			
Serum AAG Sample[w] (central lab)			X	X[y]			
Part 2 Only: Survival, Subsequent Treatments, and Secondary Malignancy Monitoring							X[z]

AAG = alpha-1 acid glycoprotein, ALP = alkaline phosphatase, ALT = alanine transaminase, ANC = absolute neutrophil count, AST = aspartate transaminase, BUN = blood urea nitrogen, CR = complete response, CT = computer tomography, ECG = electrocardiogram, HDL= high-density lipoprotein, IRR = independent radiology review, LDL = low-density lipoprotein, MCH = mean corpuscular hemoglobin, MCHC, = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, MRI = magnetic resonance imaging, PET = positron emission tomography, PK = pharmacokinetic, PR = partial response, SAE = serious adverse event, WBC = white blood cell, WOCBP = women of childbearing potential

[a] = Treatment cycles are 28 days. Unless otherwise specified, all assessments are to be completed within ± 3 days of scheduled time point.

[b] = Any procedures required on Day 1 of Cycle 1 may be omitted if completed ≤3 days earlier during the screening period.

[c] = Consent may be completed outside the 28 screening window as consent does not expire. Reconsent is not required if outside the screening window.

[d] = Patient's medical record must include prior treatments received, dates of administration, date of progression, and radiology report(s) and/or CA-125 results to support assessment of disease progression. *gBRCA* test results, if known, will also be captured.

Table 4 Schedule of Assessments

Procedure[a]	Screening Phase	Treatment Phase (±3 days of scheduled timepoint)			Post-Treatment Phase		
		Cycle 1		Cycles 2+			
	Day -28 to Day -1	Day 1[b]	Day 15	Day 1	End of Treatment	28 Day Follow-up (FU) (28 ± 3 days after last dose)	Long-term FU

[e] = A complete physical exam should be performed at Screening and End of Treatment; a limited physical exam may be performed at all other visits. Height at screening only.

[f] = Vital signs (blood pressure, pulse, and temperature) to be taken on clinic visit days.

[g] = AEs are recorded from the time of signing of informed consent through 28 days after last dose of rucaparib. Ongoing SAEs will be followed to resolution.

[h] = Heart rate, PR, QRS, QT, and rhythm. Investigator to review results and assess as normal or abnormal (clinically significant or not clinically significant). ECGs to be repeated as clinically indicated.

[i] = Includes hemoglobin, hematocrit, WBC and differential (with ANC), and platelet count (all patients in Parts 1 and 2; patients in Part 2 are also required to have MCV, MCH, and MCHC and reticulocyte count measured). Blood will be analyzed by a local laboratory and results must be reviewed by the investigator prior to dosing with rucaparib.

[j] = To be performed ≤14 days prior to the first dose of rucaparib.

[k] = Includes total protein, albumin, creatinine or estimated GFR using Cockcroft Gault formula, BUN or urea, total bilirubin, ALP, ALT, AST, total cholesterol, glucose, sodium, potassium, chloride, CO_2, calcium, and phosphorus (all patients in Parts 1 and 2; patients in Part 2 are also required to have a lipid panel that includes LDL, HDL and triglycerides in addition to the total cholesterol measurement; the lipid panel does not require fasting). Blood will be analyzed by a local laboratory and results must be reviewed by the investigator prior to dosing with rucaparib. In the event that ≥ Grade 2 AST elevations are observed, the Investigator should consider performing the following additional tests: GGT, CK/CPK, and PT.

[l] = Women of childbearing potential must have a negative serum pregnancy test result <3 days prior to the first dose of rucaparib. A serum or urine pregnancy test (investigator's discretion) must be performed <3 days prior to Day 1 of every cycle during the treatment phase. A serum pregnancy test must be performed at the End of Treatment visit.

[m] = Includes dipstick for protein, glucose, blood, pH, and ketones. If dipstick findings abnormal, perform microscopic evaluation.

[n] = Disease assessment to include clinical examination, CA-125 (if applicable), and appropriate imaging techniques, including CT scans of the chest, abdomen and pelvis, with appropriate slice thickness per RECIST; other studies (MRI, X-ray, PET, and ultrasound) may be performed if required. The same methods used to detect lesions at baseline are to be used to follow the same lesions throughout the clinical study. If a patient has known brain metastases, this disease should be evaluated at each required assessment. Copies of CT scans will be collected from all patients in Part 2 of the study and may be collected from selected patients in Part 1 of the study. Independent radiology review may be conducted on all or a subset of CT scans.

[o] = Tumor scans to be performed at the end of every 8 weeks (± 4 days) while on study. A confirmatory scan should be performed ≥4 weeks after an initial response of PR or CR is observed. Patients who have been on study at least 18 months, may decrease the frequency of disease assessments to every 16 (±2) weeks.

[p] = Disease assessments should also be done at the time of treatment discontinuation if it has been ≥8 weeks since the last assessment.

Table 4 Schedule of Assessments

Procedure[a]	Screening Phase	Treatment Phase (±3 days of scheduled timepoint)			Post-Treatment Phase		
		Cycle 1		Cycles 2+			
	Day -28 to Day -1	Day 1[b]	Day 15	Day 1	End of Treatment	28 Day Follow-up (FU) (28 ± 3 days after last dose)	Long-term FU

[q] = To be performed at the end of every 8 weeks (± 4 days) until radiologically confirmed disease progression, death or initiation of subsequent treatment for any patient who discontinued from study treatment for reason other than disease progression or death. Patients who have been on study at least 18 months may decrease the frequency of tumor scans to every 16 (±2) weeks.

[r] = To be evaluable for CA-125 response, at least 2 pretreatment samples must be collected at least 1 day, but not more than 3 months, apart. At least one sample should be within 1 week prior to the first dose of rucaparib. Both samples must be at least twice the ULN for CA-125 response assessment.

[s] = CA-125 measurement should be done on Day 1 of every cycle, at the end of treatment, and as clinically indicated.

[t] = Screening biopsy must be collected within 28 days and at least 7 days prior to first dose of study drug. Biopsy sample must be of solid tumor tissue; ascites is not acceptable. This screening sample must be sufficient for the planned analyses and deemed of adequate quality by the central laboratory prior to enrollment of patient into the study. Refer to the Pathology Charter for detailed sample handling instructions. If a biopsy was recently performed as standard of care prior to this patient consenting to this study or after study informed consent but outside the 28 day screening window this may be acceptable with advance approval from the Sponsor. *Note: the screening biopsy sample is optional for Part 2 patients known to harbor a deleterious gBRCA mutation.*

[u] = An optional post-treatment tumor biopsy sample may be collected from patients who progress on rucaparib. If the progression is due to new lesions, the preference is to obtain the biopsy from the new lesion(s). Additional consent is required. Refer to the Pathology Charter for detailed sample handling instructions.

[v] = The presence of adequate archival tissue for planned analyses must be confirmed during screening; however, shipment of archival tumor tissue is not required prior to enrollment. Refer to the Pathology Charter for detailed sample handling instructions.

[w] = Collect and process for plasma. Refer to the Laboratory Manual for detailed sample processing instructions.

[x] = A single sample should be collected as close to 12 hours after the last dose has been taken as possible and prior to the next dose. Refer to the Laboratory Manual for sample processing instructions.

[y] = Cycles 2, 3, and 4 only.

[z] = All Part 2 patients discontinued from treatment, regardless of reason, should be followed for survival, subsequent therapies, and secondary malignancy every 12 weeks until death, loss to follow-up, withdrawal of consent from study or study closure, whichever happens first. Follow-up can be performed via the telephone. Diagnosis of any secondary malignancy requires appropriate documentation (i.e., laboratory and/or pathology reports).

9.2 Screening Phase

Following written informed consent, and unless otherwise specified, the following assessments will be performed during the 28-day period prior to the first dose of study drug. Assessments performed within this window, but prior to patient signing informed consent, are acceptable only if confirmed to have been standard of care.

- Medical/oncology history, including demographic information (birth date, race, gender, etc.) and smoking status, including date of cancer diagnosis, and any surgical procedures
- Physical examination by body system, height, and weight
- ECOG performance status (Appendix C)
- Vital signs (blood pressure, pulse, and temperature)
- Prior and concomitant medications and any procedures
- 12-lead ECG
- Hematology (hemoglobin, hematocrit, WBC and differential [with ANC], and platelet count) ≤14 days prior to the first dose of study drug (all patients in Parts 1 and 2; patients in Part 2 will also have MCH, MCV, MCHC, and reticulocyte count measurements)
- Serum chemistry (total protein, albumin, creatinine or estimated GFR using the Cockcroft Gault formula, blood urea nitrogen [BUN] or urea, total bilirubin, ALP, ALT, AST, glucose, sodium, potassium, chloride, CO_2, calcium, and phosphorus) and total cholesterol for all patients in Parts 1 and 2; patients in Part 2 will also have a lipid panel that includes LDL, HDL and triglycerides in addition to the total cholesterol measurement) ≤14 days prior to the first dose of study drug. *Note: fasting is not required for the lipid panel.*
- Serum pregnancy test for women of childbearing potential (within 3 days of first dose of study drug)
- Cancer antigen 125 (CA-125) measurements per GCIG criteria provided in Appendix E
  - To be evaluable for response by CA-125, at least 2 pretreatment samples must be collected at least 1 day, but not more than 3 months, apart. At least one pretreatment sample should be within 1 week prior to the first dose of rucaparib. Both must be at least twice the upper limit of normal.
- Urinalysis performed on freshly voided clean sample (dipstick for protein, glucose, blood, pH, and ketones). If dipstick findings are abnormal based on investigator judgment, then a microscopic evaluation will be performed to assess the abnormal findings.
- Tumor assessments should consist of clinical examination and appropriate imaging techniques, including CT scans of the chest, abdomen, and pelvis with appropriate slice thickness per RECIST; other studies (MRI, X-ray, positron emission tomography [PET], and ultrasound) may be performed if required. The same methods used to detect lesions at baseline are to be used to follow lesions throughout the clinical study. If a patient has known brain metastases, this disease should be evaluated at each required assessment.

- Tumor tissue biopsy / sample collection (If a biopsy was recently performed as standard of care prior to this patient consenting to this study or after study informed consent but outside the 28 day screening window this may be acceptable with advance approval from the Sponsor). Tumor specimen must be processed locally as FFPE tissue. Sample must be sent to the central laboratory for review and confirmed as adequate (at least 20% tumor content with a minimum of 80% nucleated cellular content; 30% or greater tumor content is preferred) for planned analyses prior to enrollment. Refer to the Pathology Charter for detailed sample requirements and handling instructions.
  - To ensure sufficient viable tumor tissue is obtained, image-guided biopsies should be achieved with 14 to 18 gauge cutting needles to provide 1 to 3 cores measuring 1 to 1.5 cm in length.
  - Biopsy must be of solid tumor tissue; ascites is not acceptable.
  - If tumor tissue obtained from the biopsy is deemed not adequate, and the patient is unwilling or unable to have another biopsy, the patient may be considered for enrollment if archival tumor tissue is provided and deemed of adequate quality. This must occur prior to any treatment with rucaparib.
  - Note: screening biopsy sample is optional for Part 2 patients known to harbor a deleterious *gBRCA* mutation
- FFPE archival tumor tissue sample. Sufficient archival FFPE tumor tissue for planned analyses should be provided. Cytospin blocks from ascites are not acceptable. Refer to the Pathology Charter for detailed sample requirements and handling instructions.
  - The most recently obtained tumor tissue that is of adequate quality (at least 20% tumor content with a minimum of 80% nucleated cellular content) should be submitted
  - Sample need not be submitted prior to enrollment; however, confirmation that such tissue is available must be provided prior to enrollment approval.
- AE monitoring (after signing informed consent)
- Blood sample for ctDNA analysis
- Blood sample for storage

9.3 Treatment Phase

9.3.1 Day 1 of Cycle 1

The following procedures will be completed before rucaparib is administered:

- Physical examination (abbreviated)
- Weight
- ECOG performance status (Appendix C)
- Vital signs
- Concomitant medications and procedures

- Hematology
- Serum chemistry
- CA-125 measurement
- AE monitoring
- Blood sample for ctDNA analysis

Rucaparib tablets will be dispensed to the patient in sufficient quantity to last until the next treatment cycle. Patients will ingest rucaparib twice daily at about the same times every day, at close to 12 hours apart as possible. Each dose of rucaparib should be taken with at least 8 oz (240 mL) of room temperature water. Patients may take rucaparib on an empty stomach or with food. Patients will record dosing information in their dosing diary.

9.3.2 Day 15 of Cycle 1

Patients will be instructed to refrain from taking their first dose of rucaparib at home on the day of their clinic visit because certain assessments should be performed prior to dosing.

- Hematology
- Serum chemistry
- A single plasma PK sample (as close to 12 hours after the last dose taken as possible and prior to the next dose)
- A single serum sample for AAG measurement (as close to 12 hours after the last dose taken as possible and prior to the next dose)
- Concomitant medications and procedures
- AE monitoring

9.3.3 Day 1 of Cycles 2 and Beyond

Patients should be instructed to refrain from taking their first dose of rucaparib at home on Day 1 of Cycles 2, 3 and 4. On these days the blood samples for PK and AAG measurement must be drawn prior to the first dose administered.

The following procedures will be completed on Day 1 of Cycles 2 and beyond:

- Physical examination (abbreviated)
- Weight
- ECOG performance status (Appendix C)
- Vital signs
- Concomitant medications and procedures
- Hematology

- Serum chemistry
- CA-125 measurement should be done on Day 1 of every cycle and as clinically indicated.
- Serum or urine pregnancy (per investigator's discretion) <3 days prior to start of cycle (for women of childbearing potential only)
- Tumor scans (using the same methodology as was used at screening) at the end of every 8 weeks (±4 days) after initiation of treatment. Patients who have been on study at least 18 months, may decrease the frequency of disease assessments to every 16 (±2) weeks.
- Blood sample for ctDNA analysis
- A single plasma PK sample (as close to 12 hours after the last dose taken as possible and prior to the next dose) (Day 1 of Cycles 2, 3, and 4 only)
- A single serum sample for AAG measurement (as close to 12 hours after the last dose taken as possible and prior to the next dose) (Day 1 of Cycles 2, 3, and 4 only)
- AE monitoring
- Study drug accountability

Rucaparib tablets will be dispensed to the patient in sufficient quantity to last until the next clinic visit. A single dose of rucaparib will be administered with at least 8 oz (240 mL) of room temperature water during the current clinic visit. Patients may take rucaparib on an empty stomach or with food. Patient will record dosing information in their dosing diary.

Patients will continue dosing with rucaparib at home on an empty stomach or with food, taking doses twice daily at about the same times every day. Rucaparib should be taken with at least 8 oz (240 mL) of room temperature water. Patient will record dosing information in the dosing diary.

9.4 End of Treatment Visit

The following procedures will be performed for all patients as soon as possible after the last dose of rucaparib:

- Physical examination
- Weight
- ECOG performance status (Appendix C)
- Vital signs
- Concomitant medications and procedures
- 12-lead ECG
- Hematology
- Serum chemistry
- Serum pregnancy test for women of childbearing potential

- CA-125 measurement
- Blood sample for ctDNA analysis
- Optional tumor tissue biopsy sample collection at time of disease progression/treatment discontinuation (requires additional consent). If disease progression is caused by appearance of a new lesion(s), this lesion should be prioritized for biopsy. Tumor tissue will be processed locally as FFPE tissue. Refer to the Pathology Charter for detailed sample handling instructions.
- AE monitoring
- Study drug accountability
- Disease assessments should also be done at the time of treatment discontinuation if it has been ≥8 weeks since the last assessment.

9.5 28 Day Follow-up Visit

The following procedures will be performed for all patients at 28 (±3) days after the last dose of rucaparib:

- AE monitoring (ongoing SAEs should be followed until resolution or stabilization)

9.6 Long-term Follow-up

Disease assessment will be completed for all patients who discontinued treatment for reason other than disease progression or death. Tumor scans should continue to be performed at 8 (±4 days) week intervals until confirmed radiologic disease progression, death or initiation of subsequent therapy. Patients who have been on study at least 18 months, may decrease the frequency of tumor scans to every 16 (±2) weeks.

All patients in Part 2 will be followed for survival, subsequent therapy, and secondary malignancy every 12 weeks until death, loss to follow-up, withdrawal of consent from study or study closure, whichever happens first. Follow-up can be performed via the telephone. Diagnosis of any secondary malignancy requires appropriate documentation (i.e., laboratory and/or pathology reports). Follow-up can be performed via the telephone. Diagnosis of any secondary malignancy requires appropriate documentation (i.e., laboratory and/or pathology reports).

9.7 Methods of Data Collection

Hematology, serum chemistry (including CA-125), serum/urine pregnancy, and urinalysis will be performed locally. Central/core laboratories will conduct all other assays described below. Please refer to the Pathology Charter and/or Laboratory Manual for details on collecting and processing all samples that will be sent to central/core laboratories.

9.7.1 Pharmacokinetic Evaluations and AAG Measurement

For all patients, 4 mL blood samples for trough level PK analysis of rucaparib will be drawn on Day 15 of Cycle 1 and on Day 1 of Cycles 2, 3, and 4, prior to dosing with rucaparib and as close to 12 hours after the last dose was taken as possible.

Serum samples for AAG analysis will be collected pre-dose on the same days as PK blood samples.

9.7.2 Biomarker Analysis – FFPE Tumor Tissue

A tumor tissue biopsy sample is required to be collected during screening from all patients, except for Part 2 patients known to harbor a deleterious *gBRCA* mutation; the screening biopsy sample in this group of patients is optional. A tumor tissue biopsy sample at the time of disease progression/treatment discontinuation is optional; patients must provide additional consent for this optional tumor tissue biopsy sample. If disease progression is caused by appearance of a new lesion(s), this lesion should be prioritized for the optional biopsy.

Sufficient archival FFPE tumor tissue (See Pathology Charter for details) must be available and located during the screening process and submitted to the central laboratory as soon as possible. Submission of archival tumor tissue is not required for enrollment; however, confirmation that such tissue is available is required.

Analysis of the tumor tissue samples may include, but not be limited to:

- DNA extraction and sequencing of single nucleotide polymorphisms (SNPs) to identify tumor genomic LOH and to determine whether tumor genomic LOH can be used as a predictor of efficacy
- DNA extraction and sequencing in order to identify:
- If the patient has a mutation in BRCA1, BRCA2, or another gene in the HRR pathway (Appendix A)
- If a patient has a BRCA reversion or other mutation(s) that may be associated with efficacy
- Gene expression profiling on extracted RNA to potentially identify a signature associated with efficacy
- Immunohistochemistry analysis to assess NHEJ pathway integrity

9.7.3 Biomarker Analysis – Blood

ctDNA Analysis: Up to 10 mL of whole blood for ctDNA analysis will be collected at screening, on Day 1 of each cycle, and at the End of Treatment visit.

A blood sample collected at screening will also be stored. Prior to final analysis, genomic DNA may be analyzed in an exploratory fashion in order to determine whether the mutation is germline or somatic.

9.7.4 Safety Evaluations

9.7.4.1 Adverse Event Assessment

The investigator has the responsibility for assessing the safety of the patients and for compliance with the protocol to ensure study integrity. Patients will be monitored for AEs during study participation (beginning at the time informed consent is obtained) and until 28 days after the last dose of rucaparib. Any ongoing SAEs will be followed until resolution or stabilization. AEs and laboratory abnormalities will be graded according to the NCI CTCAE grading system (Version 4.0) and recorded on the eCRF.

Complete details for monitoring AEs, including the definition of drug-related AEs, are provided in Section 10.

9.7.4.2 Clinical Laboratory Investigations

Certified local laboratories will perform study-related clinical laboratory tests according to institutional procedures, and the results will be reviewed by the investigator. The panels of laboratory tests to be performed are shown below:

Hematology: Hemoglobin, hematocrit, WBC and differential (with ANC), and platelet count at Screening, during treatment, and at the End of Treatment visit for all patients in Parts 1 and 2. In addition, MCV, MCH, MCHC, and reticulocyte count will also be assessed for all patients in Part 2. Hematology results must be reviewed by the investigator prior to the start of treatment with study drug.

Clinical Chemistry: Total protein, albumin, creatinine or estimated GFR using the Cockcroft Gault formula, BUN or urea, total bilirubin, alkaline phosphatase (ALP), ALT, AST, total cholesterol, glucose, sodium, potassium, chloride, CO_2, calcium, and phosphorus at Screening, during treatment, and at the End of Treatment visit for all patients in Parts 1 and 2. A lipid panel that includes LDL, HDL, and triglycerides in addition to total cholesterol will also be assessed for all patients in Part 2. Fasting is not required for the lipid panel. Clinical chemistry results must be reviewed by the Investigator prior to the start of treatment with study drug. In the event that ≥ Grade 2 AST elevation is observed, the Investigator should consider performing the following additional tests: GGT, CK/ CPK, and PT.

Urinalysis: Performed on freshly voided clean sample by dipstick for protein, glucose, blood, pH, and ketones per the schedule of evaluations. If dipstick findings are abnormal, then a microscopic evaluation will be performed to assess the abnormal findings. Urinalysis will be performed at screening only.

Serum/Urine Pregnancy: For women of childbearing potential only. Serum pregnancy to be performed <3 days prior to first dose of rucaparib and at End of Treatment. Serum or urine pregnancy (per investigator's discretion) to be performed <3 days prior to the start of every cycle during the treatment phase.

Laboratory reports will be reviewed by the investigator or delegated physician who will then comment on out-of-range parameters and assess clinical significance. Clinically significant abnormalities and associated panel results, as well as results of any additional tests performed as follow-up to the abnormalities, will be documented on the eCRF as an AE. Refer to Section 10.4 for guidelines on reporting of abnormal laboratory values as AEs.

9.7.4.3 Vital Signs

Vital signs will include blood pressure, pulse, and body temperature. Vital signs will be performed at most study visits.

9.7.4.4 12-Lead Electrocardiograms

For all patients, 12-lead ECGs will be taken at the following time points:

- Screening (within 28 days prior to first rucaparib dose)
- End of Treatment

The 12-lead ECGs will be analyzed locally.

9.7.4.5 Body Weight and Height

Height will be measured during the Screening visit only. Weight will be measured per institutional guidelines at Screening, on Day 1 of each cycle, and at the End of Treatment visit.

9.7.4.6 Physical Examinations

Physical examinations will include an assessment of all the major body systems. Physical examinations will be performed at Screening and End of Treatment (complete) and at most study visits (limited as appropriate).

9.7.4.7 ECOG Performance Status

ECOG performance status (Appendix C) will be assessed at Screening, on Day 1 of each cycle, and at the End of Treatment visit. ECOG performance status should be assessed by the same study personnel at each visit, if possible. Care will be taken to accurately score performance status, especially during screening for study eligibility purposes. Additional consideration should be given to borderline ECOG performance status to avoid enrolling patients with significant impairment.

9.7.5 Efficacy Evaluations

9.7.5.1 Tumor Assessments

Tumor assessments will be performed at Screening, at the end of every 8 weeks (±4 days), and post-treatment (if patient discontinued treatment for any reason other than radiologically confirmed disease progression) until radiologically confirmed disease progression, death or initiation of subsequent treatment. Patients who have been on study at least 18 months, may

decrease the frequency of tumor assessments to every 16 (±2) weeks. Tumor assessments should be done at the time of treatment discontinuation if it has been ≥8 weeks since the last assessment. A confirmatory scan should be performed at least 4 weeks after an initial response of PR or CR is observed. Tumor response will be interpreted using RECIST Version 1.1 (Appendix B).

Tumor assessments should consist of clinical examination and appropriate imaging techniques (CT scans of the chest, abdomen, and pelvis with appropriate slice thickness per RECIST); other studies (MRI, X-ray, PET, and ultrasound) may be performed if required. If a patient has known brain metastases, this disease should be evaluated at each required assessment. The same methods used to detect lesions at baseline are to be used to follow the same lesions throughout the clinical study. Investigators should perform scans of the anatomical sites that, in their judgment, are appropriate to assess based on each patient's tumor status.

Copies of CT scans will be collected from all patients in Part 2 of the study and may be collected from selected patients in Part 1 of the study. Independent radiology review may be conducted on all or a subset of CT scans.

9.7.5.2 Tumor Markers

CA-125 will be collected at Screening, on Day 1 of every cycle, at the End of Treatment visit, and as clinically indicated.

10 ADVERSE EVENT MANAGEMENT

10.1 Definition of an Adverse Event

An AE is any untoward medical occurrence, including the exacerbation of a pre-existing condition, in a patient administered a pharmaceutical product. The pharmaceutical product does not necessarily have a causal relationship with the AE. Anticipated fluctuations of pre-existing conditions, including the disease under study, that do not represent a clinically significant exacerbation or worsening are not considered AEs.

For the purposes of this study, disease progression of the patient's tumor with new or worsening symptoms must be documented as an AE. However, disease progression documented solely by radiographic evidence with no new or worsening symptoms will not require reporting as an AE.

It is the responsibility of the investigator to document all AEs that occur during the study. AEs should be elicited by asking the patient a nonleading question (e.g., "Have you experienced any new or changed symptoms since we last asked/since your last visit?"). AEs will be reported on the AE eCRF. Symptoms reported spontaneously by the patient during the physical examination will also be documented on the AE eCRF.

10.2 Definition of a Serious Adverse Event

An SAE is any untoward medical occurrence that occurs at any dose (including after informed consent is given and prior to dosing) that:

- Results in death.
- Is immediately life-threatening (i.e., the patient is at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe).
- Requires in-patient hospitalization or prolongation of existing hospitalization.
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Results in a congenital anomaly or birth defect.
- Is an important medical event based upon appropriate medical judgment; it may jeopardize the patient or may require intervention to prevent one of the other outcomes noted above.

10.3 Exceptions to Serious Adverse Event Reporting

The following are not considered SAEs and therefore are not required to be reported to the Sponsor:

- Pre-planned or elective hospitalization, including social and/or convenience situations (e.g., respite care).

- Overdose of study drug or concomitant medication, unless there is an AE that meets SAE criteria (e.g., hospitalization), as a direct consequence of the overdose. This should be entered as Overdose - followed by the appropriate AE/SAE term.
- Progression of the patient's underlying cancer (disease progression) documented solely on radiographic evidence with no new or worsening symptoms.

10.4 Clinical Laboratory Assessments and Other Abnormal Assessments as Adverse Events and Serious Adverse Events

It is the responsibility of the Investigator to assess the clinical significance of all abnormal laboratory values as defined by the list of reference ranges from the local laboratory. In some cases, significant change in laboratory values within the normal range may require similar assessment.

An abnormal value that is not already associated with an AE is to be recorded as an AE only if one of the following criteria is met:

- It resulted in treatment modification (reduction of dose, interruption of dosing, or permanent discontinuation of study drug)
- It required intervention / management
- It is suggestive of organ toxicity
- The Investigator considers it to be clinically significant

10.5 Pregnancy or Drug Exposure During Pregnancy

If a patient becomes pregnant during the course of the study, study drug dosing should be held immediately.

Pregnancy is not considered to be an AE or SAE; however, all pregnancies must be reported to the Sponsor using the Clinical Pregnancy Report form within the same timelines as for as SAE.

All pregnancies should be followed through to outcome whenever possible. Once the outcome of a pregnancy is known, the Clinical Pregnancy Outcome Report form should be completed and submitted to the Sponsor.

10.6 Recording of Adverse Events and Serious Adverse Events

All AEs, serious and non-serious, will be fully documented on the appropriate eCRF. For each AE, the Investigator must provide duration (start and end dates or ongoing), intensity, relationship to study drug, and indicate whether specific action or therapy was required.

Any AE/SAE that occurs from the time informed consent is obtained until 28 days after last dose of study drug administration will be collected, documented and reported to the Sponsor by the Investigator according to the specific definitions and instructions detailed within this protocol, whether dosing has occurred or not. After the 28-day window, only SAEs assessed as related to

study drug should be reported. If a patient is determined to be a screen failure, no further AEs/ SAEs are required to be reported once that determination has been made, with the exception of AEs/SAEs deemed related to a protocol-specified procedure.

All SAEs, regardless of relationship to study drug, must be reported to the Sponsor/designee within 24 hours of the Investigator's knowledge. This should be done by faxing or emailing the completed SAE report to the Sponsor/designee contact provided on the SAE report form.

Investigators must follow patients with SAEs until the event has resolved or the condition has stabilized. If the patient is lost to follow-up with an ongoing SAE, this should be captured accordingly on a follow-up SAE report.

10.6.1 Intensity of Adverse Events

Severity refers to the intensity of an AE. The severity of each AE will be categorized using the NCI CTCAE, Version 4.0 (http://evs.nci.nih.gov/ftp1/CTCAE/Archive/CTCAE_4.0_2009-05-29_QuickReference_8.5x11.pdf).[49]

Severity ≠Serious

For any term that is not specifically listed in the CTCAE, intensity should be assigned a grade of 1-5 using the following CTCAE guidelines:

- Mild (Grade 1): mild or asymptomatic symptoms; clinical or diagnostic observations only; intervention not indicated
- Moderate (Grade 2): limiting age-appropriate instrumental activities of daily living; minimal, local or noninvasive intervention indicated
- Severe (Grade 3): limiting self-care activities of daily living; hospitalization indicated
- Life threatening (Grade 4): life-threatening consequences; urgent intervention indicated
- Fatal (Grade 5): results in death

10.6.2 Causal Relationship of Adverse Events to Investigational Product

Medical judgment should be used to determine the cause of the AE considering all relevant factors such as but not limited to: the disease under study, concurrent disease, concomitant

medication, relevant history, pattern of the AE, temporal relationship to the study medication, dechallenge or rechallenge.

Not Related To Study Drug	An AE that is clearly due to extraneous causes (e.g., concurrent disease, concomitant medication, disease under study, etc.) An AE that does not follow a reasonable temporal sequence from administration of the study drug. An AE that does not reappear or worsen when study drug is restarted. An AE for which an alternative explanation is likely, but not clearly identifiable.
Related to Study Drug	An AE that is difficult to assign to alternative causes. An AE that follows a strong or reasonable temporal sequence from administration of study drug. An AE that could not be reasonably explained by the patient's clinical state, concurrent disease, or other concomitant therapy administered to the patient. An AE that is confirmed with a positive rechallenge or supporting laboratory data.

10.6.3 Outcome

The investigator will record the outcome for each AE according to the following criteria:

Outcome

- Recovered/Resolved
- Recovered/Resolved with sequelae
- Improving
- Ongoing
- Death
- Unknown/Lost to follow-up

10.7 Regulatory Aspects of Adverse Event Reporting

All SAEs, regardless of relationship to study drug, must be reported to the Sponsor's SAE designee within 24 hours of knowledge of the event, according to the procedures below. It is important that the investigator provide an assessment of relationship of the SAE to study treatment at the time of the initial report. The Clinical Trial SAE Report Form must be used for reporting SAEs. The contact information for reporting of SAEs can be found on the SAE Reporting Form.

Clovis Oncology, Inc. (Clovis Oncology), or its designee is responsible for submitting reports of AEs associated with the use of the drug that are both serious and unexpected to FDA, according to 21 Code of Federal Regulations (CFR) 312.32, to the European regulatory authorities according to the European Commission Clinical Trials Directive (2001/20/EC); and to other regulatory authorities, according to national law and/or local regulations. All investigators participating in ongoing clinical studies with the study medication will receive copies of these

reports for prompt submission to their IRB or IEC. In accordance with the European Commission Clinical Trials Directive (2001/20/EC), Clovis Oncology or its designee will notify the relevant ethics committees in concerned member states of applicable suspected unexpected serious adverse reactions (SUSARs) as individual notifications or through periodic line listings.

Clovis Oncology or its designee will submit all safety updates and periodic reports to the regulatory authorities as required by applicable regulatory requirements.

11 STATISTICAL METHODS

11.1 Analysis Populations

The following analysis populations are defined for the study:

Safety Population – The safety population will consist of all patients who received at least one dose of protocol-specified treatment.

Efficacy Population – The efficacy population will consist of all patients evaluable for response by RECIST (Appendix B) and/or GCIG CA-125 criteria (Appendix E). Patients evaluable for a RECIST response must have at least one measureable target lesion at baseline and at least one post-baseline tumor assessment. Patients evaluable for GCIG CA-125 response must have 2 pretreatment CA-125 values at least twice the upper limit of normal and at least 2 post-baseline values.

11.2 Statistical Methods

11.2.1 General Considerations

Data will be summarized separately for Parts 1 and 2 and may also be pooled as appropriate.

The summary tables will be presented for all treated patients and by the subgroups defined by HRD status.

Quantitative variables will typically be summarized using frequencies and percentages for appropriate categorizations and may also be summarized using descriptive statistics. For variables summarized with descriptive statistics, the following will be presented: N, mean, standard deviation, median, minimum and maximum. Categorical variables will be presented using frequencies and percentages. The Kaplan-Meier methodology will be used to summarize time-to-event variables. If estimable, the 25th, 50th (median), and 75th percentiles will be presented along with the Kaplan-Meier estimates of event rates at 6-month intervals. The number of patients with events and the number of censored patients will also be presented.

All data will be used to their maximum possible extent but without any imputations for missing data.

All statistical analyses will be conducted with the SAS® System, version 9.3 or higher.

Unless otherwise specified, baseline is defined as the last measurement on or prior to the first day of study drug administration.

11.2.2 Patient Disposition

Patient disposition (analysis population allocation, entered, discontinued, along with primary reason for discontinuation) will be summarized using frequency counts, and the corresponding percentages.

11.2.3 Baseline Characteristics

All demographic and baseline characteristics will be summarized for the safety population.

The following variables will be summarized with frequency tabulations:

- Time since diagnosis (months): > 6-12, > 12-24, > 24;
- Baseline laboratory parameters: graded based on CTCAE;

Descriptive statistics may also be used to summarize these variables.

11.2.4 Efficacy Analyses

All primary and secondary efficacy evaluations will be presented by HRD status and study part (Part 1 or Part 2). Data may also be pooled across HRD status or Parts 1 and 2 as appropriate. Analyses of PFS will be presented for the safety population and ORR and CA-125 response rates will be presented for the appropriate subset of the efficacy evaluable population.

11.2.4.1 Primary Efficacy Analyses

Part 1: The primary efficacy endpoint of PFS will be calculated as 1+ the number of days from the first dose of study drug to disease progression, as determined by the investigator or death due to any cause, whichever occurs first, in molecularly defined subgroups. Patients without a documented event of progression will be censored on the date of their last adequate tumor assessment (i.e., radiologic assessment) or date of first dose of study drug if no tumor assessments have been performed.

Part 2: The primary efficacy endpoint of ORR is defined as the proportion of patients with a best response of CR or PR using RECIST v1.1 (Appendix B) as assessed by the Investigator. The ORR will be summarized with frequencies and percentages in the efficacy population.

Independent radiology review may also be performed as a supportive analysis for all or a subset of patients. The supportive analysis of ORR by independent radiology review (irrORR) is defined as the proportion of patients with a best response of CR or PR using RECIST v1.1 (Appendix B) as assessed by independent radiology review.

11.2.4.2 Secondary Efficacy Analyses

11.2.4.2.1 Objective Response Rate (ORR) (Part 1)

ORR is defined as the proportion of patients with a best response of CR or PR using RECIST v1.1 (Appendix B) as assessed by the Investigator. The ORR will be summarized with frequencies and percentages.

As a supportive analysis, the ORR will also be evaluated in the safety population. Patients who are not evaluable for a RECIST response will be considered to have experienced disease progression.

Independent radiology review may also be performed as a supportive analysis. The supportive analysis of ORR by independent radiology review (irrORR) is defined as the proportion of patients with a best response of CR or PR using RECIST v1.1 (Appendix B) as assessed by independent radiology review. The irrORR will be evaluated in the efficacy population for patients with measurable disease per the independent radiology review.

11.2.4.2.2 Duration of Response

The duration of response is measured from the time measurement criteria are met for CR/PR per RECIST or a 50% response in CA-125 (whichever is first recorded) until the first date that recurrent or PD is objectively documented using the earliest of the RECIST or CA-125 response.

The duration of response will also be evaluated separately for CR/PR RECIST responses and for CA-125 responses. In addition, the duration of overall CR will be measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

The duration of response will be summarized with descriptive statistics. Only patients with a response will be included in the summary.

11.2.4.2.3 ORR Assessed by RECIST and GCIG CA-125 Criteria

The endpoint of ORR defined as a best response of CR or PR using RECIST (Appendix B) will be summarized with frequencies and percentages in the appropriate efficacy population.

The endpoint of CA-125 response rate defined as a 50% reduction in CA-125 as assessed by GCIG criteria will be summarized with frequencies and percentages in the safety population. As a supportive analysis, the CA-125 response rate will also be evaluated in the patients evaluable for a CA-125 response as defined in Appendix E.

ORR will be reported separately and together for RECIST and GCIG. The combined ORR will be assessed as indicated in Table 5.

Table 5 Overall Response by RECIST[50] and GCIG CA-125 Criteria[51]		
RECIST Response	**GCIG CA-125 Response**	**RECIST + GCIG CA-125 Combined**
CR (requires normalization of CA-125)	CA-125 within normal range	Response
PR	Response	Response
PR	No Response	Response
SD	Response	Response
SD	No Response	No Response
PD	Response	No Response
PD	No Response	No Response

11.2.4.2.4 Overall Survival (Part 2)

Overall survival (OS) is defined as the number of days from the date of first dose of study drug to the date of death (due to any cause). Patients without a known date of death will be censored on the date the patient was last known to be alive.

11.2.4.3 Exploratory Efficacy Analyses

Statistical analysis of exploratory endpoints will be detailed in the Statistical Analysis Plan.

11.2.4.4 Diagnostic Test

The predictive utility of the HRD diagnostic test will be evaluated by comparing the primary and secondary endpoints in the t*BRCA* subgroup to that of nbHRD subgroup and the biomarker negative subgroup.

11.2.5 Pharmacokinetic Analyses

As a secondary endpoint of the study, trough (C_{min}) concentrations of rucaparib will be summarized with descriptive statistics overall and by cycle in all patients with at least one PK sample collected.

11.2.6 Safety Analyses

The safety analyses will be performed using the safety population (all patients who have received at least one dose of rucaparib).

11.2.6.1 Adverse Events

Adverse events will be classified using the Medical Dictionary for Drug Regulatory Activities (MedDRA) classification system. The severity of the toxicities will be graded according to the NCI CTCAE whenever possible. Treatment-emergent adverse events (TEAEs) are defined as AEs with onset date on or after the date of first dose of study medication until the date of the last study medication dose plus 28 days. Adverse events will be considered treatment-emergent if all or part of the date of onset of the adverse event is missing and it cannot be determined if the adverse event meets the definition for treatment-emergent.

The number and percentage of patients who experienced TEAEs for each system organ class and preferred term will be presented. Multiple instances of the TEAE in each system organ class and multiple occurrences of the same preferred term are counted only once per patient. The number and percentage of patients with at least one TEAE will also be summarized.

Separate tables will be presented as follows:

- All TEAEs;
- TEAEs by CTCAE grade;
- Grade 3 or greater TEAEs;
- Treatment-related TEAEs;
- Serious TEAEs;
- TEAEs with an outcome of death;
- TEAEs leading to discontinuation of study medication;
- TEAEs resulting in interruption/delay of study medication; and
- TEAEs resulting in reduction of study medication.

The incidence of TEAEs will be summarized by relationship to study drug according to the following categories: "treatment-related," or "not treatment-related". The category of treatment-related contains the TEAEs with a missing relationship. If a patient experiences multiple occurrences of the same AE with different relationship categories, the patient will be counted once, as a relationship category of treatment related.

If a patient experiences multiple occurrences of the same AE with different toxicity grades, the patient will be counted once for the maximum (most severe) toxicity grade. AEs with a missing toxicity grade will be presented in the summary table with a toxicity grade of "Missing." For each toxicity grade, the number and percentage of patients with at least one TEAE of the given grade will be summarized.

Non-TEAEs (pre-treatment and post-treatment) will be presented in the by patient data listings for the safety population.

11.2.6.2 Clinical Laboratory Evaluations

Clinical laboratory evaluations include the continuous variables for hematology, serum chemistry, and urinalysis. The laboratory values will be presented in SI units. The on-treatment period will be defined as the time from enrollment to 28 days after the last dose of study drug. Laboratory values collected during the on-treatment period will be included in the summary tables. The laboratory values collected after the on-treatment period will only be presented in the data listings.

The summary of laboratory data will include descriptive statistics (N, mean, SD, minimum, median, and maximum) of the maximum, minimum and last value during the on-treatment period. Summaries using descriptive statistics of the change from baseline to the maximum, minimum, and last value during the on-treatment period will also be given.

Supporting laboratory data including normal ranges and abnormal laboratory flags will be provided using by-patient listings. Separate listings will be produced for clinically significant laboratory abnormalities (i.e., those that meet Grade 3 or 4 criteria according to CTCAE Version 4.0).

11.2.6.3 Vital Sign Measurements

The on-treatment period will be defined as the time from enrollment to 28 days after the last dose of study drug. Vital sign measurements collected during the on-treatment period will be included in the summary tables. The vital sign measurements collected after the on-treatment period will only be presented in the data listings.

The summary of vital sign data will include descriptive statistics (N, mean, SD, minimum, median, third quartile and maximum) of the maximum, minimum and last value during the on-treatment period. Summaries using descriptive statistics (N, mean, SD, minimum, median and maximum) of the change from baseline to the maximum, minimum, and last value during the on-treatment period will also be given.

11.3 Interim Analyses

No formal interim efficacy analyses will be performed.

A formal safety data review will occur after the first 20 patients have been enrolled, then quarterly until Part 1 of the study is fully enrolled, and then every 6 months thereafter. The review committee will include external experts and Sponsor personnel. The external experts will include, but not be limited to, the coordinating PIs of the study (Dr. Elizabeth Swisher at Univ. of Washington and Dr. Iain McNeish at Univ. of Glasgow). Clovis reviewers will include the Medical Monitor, Chief Medical Officer, Head of Pharmacovigilance, and Biostatistician. The protocol will be amended as appropriate to incorporate additional patient safety monitoring if new safety signals are noted at any review.

11.4 Sample Size Considerations

The total enrollment planned for this study is approximately 480 patients, N=180 in Part 1 and up to N=300 in Part 2.

Part 1: It is anticipated that approximately 180 patients will be required in order to ensure each subgroup of patients (tBRCA, nbHRD, and biomarker negative) will contain an adequate number of patients. Other than the cap on patients with a known deleterious *gBRCA* mutation, (n=15), there will be no specific requirement to enroll defined numbers of patients into each planned subgroup. The likely size of each subgroup has been estimated based on: a) frequencies of HRD-associated genetic abnormalities at initial diagnosis as reported in the literature and b) the hypothesis that the inclusion criterion of sensitivity to platinum following the most recent line of platinum therapy will enrich the population for patients with tumors harboring mutations of HRD pathway genes (i.e., that the frequency will be greater than that described in the newly-diagnosed population). Table 6 provides estimated HRD subgroup sizes in Part 1 of this trial.

Table 6 Estimated HRD Subgroup Sizes[a]

HRD Subgroup	Expected Frequency at Diagnosis	Estimated Frequency with Enrichment for Platinum Sensitivity	Estimated Number of Patients
tBRCA	21%	30%	15 with known deleterious *gBRCA* mutation (fixed) ***plus*** 20 – 25 with somatic *BRCA* mutation ***plus*** 5 – 25 additional with newly diagnosed *gBRCA* mutation
nbHRD	22 – 32%	30 – 50%	50 – 90
Biomarker Negative	60 – 70%	20 – 40%	36 – 72

[a]Expected frequency estimates are from TCGA[1]

Enrollment of patients known a priori to harbor a *gBRCA* mutation classified as deleterious (pathogenic), suspected deleterious, or favor deleterious (or the equivalent interpretation of any of these) on the most recent assessment by a testing laboratory will be limited to 15 in Part 1. Fifteen patients with a known *gBRCA* mutation are sufficient to establish that the frequency of *gBRCA* mutation reversions is low. In particular, if none of the 15 patients with a known *gBRCA* mutation is shown to have a reversion between archival tissue and tumor tissue collected at screening, then the frequency of *gBRCA* reversions is likely less than 20% as the upper bound on the 90% confidence interval (CI) is 18%. Additional patients, previously untested or tested and found to be $gBRCA^{wt}$, may be identified as having a *BRCA* mutation in tumor tissue, therefore the BRCA subgroup will likely contain at least 40 patients.

The benefit of rucaparib is expected to be the greatest in patients in the t*BRCA* subgroup, followed by patients in the nbHRD subgroup, and lowest in patients in the biomarker negative subgroup. This study will provide evidence as to whether the benefit of rucaparib is clinically meaningful in each of these subgroups, and particularly in the nbHRD subgroup.

With a total of 180 patients enrolled in Part 1 of the study, the comparison of any 2 subgroups will likely contain about 100 patients. Therefore with 100 patients, there is 80% power at a 2-sided 10% significance level to detect a difference in PFS distributions assuming the hazard ratio between 2 subgroups is 0.50.

Part 2: The objective of Part 2 is to estimate the ORR in each of the HRD subgroups in a more heavily pre-treated patient population (at least 3, but no more than 4, prior chemotherapy regimens). Up to 300 patients will be enrolled in Part 2 of the study in order to enroll at least 80 patients in each HRD subgroup. A total of 300 patients should be sufficient assuming an approximate 33.3% allocation to each HRD subgroup in the enrollment population.

Currently, there are few clinical studies that have prospectively evaluated response to treatment beyond the 3rd-line setting; however, retrospective analyses of patients in 3rd relapse and beyond indicate they have a short PFS (approximately 4-6 months) and OS (approximately 5-6 months).[23] Overall, there is a need for new treatments and alternatives to chemotherapy for heavily pre-treated ovarian cancer patients with advanced, relapsed disease to be explored in prospectively designed trials.

The table below provides 95% CIs for observed response rates ranging from 10 to 60% assuming a total of 80 patients within each HRD subgroup.

Confidence Intervals for Objective Response Rates (ORR)

ORR(%)	[95% CI]
10	4.4, 18.8
20	11.8, 30.4
30	20.3,41.3
40	29.2,51.6
50	38.6, 61.4
60	48.4, 70.8

CI=Confidence intervals of ORR using Clopper-Pearson methodology.[24]

An ORR ≥20% in any subgroup would be worthy of further exploration in that population.

12 PATIENT DISPOSITION

12.1 Patient Discontinuations

A patient must be discontinued from protocol-prescribed therapy if any of the following apply:

- Consent withdrawal at the patient's own request or at the request of their legally authorized representative
- Progression of patient's underlying disease
- Any event, adverse or otherwise, that, in the opinion of the investigator, would pose an unacceptable safety risk to the patient
- An intercurrent illness that, in the opinion of the Investigator, would affect assessments of the clinical status to a significant degree and requires discontinuation of therapy
- A positive pregnancy test at any time during the study
- In addition, the sponsor may discontinue the trial early for any of the reasons noted in Section 13.6.

The sponsor (or designee) should be notified of all study terminations as soon as possible. The date and reason for cessation of rucaparib must be documented in the eCRF and source documents. To the extent possible, the End of Treatment visit procedures should be performed on all patients who receive rucaparib as soon as possible following the last dose of rucaparib. Patients will be followed for 28 (±3) days after the last dose of rucaparib for safety; those with ongoing SAEs will be followed until either resolution or stabilization has been determined. Patients that discontinue treatment due to anything other than disease progression or death will be followed for tumor assessments until radiologic disease progression is confirmed, death or the initiation of new treatment.

13 STUDY ADMINISTRATION

13.1 Regulatory and Ethical Considerations

This study will be conducted in compliance with the protocol; Good Clinical Practices (GCPs), including ICH Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines; FDA regulatory requirements; and in accordance with the ethical principles of the Declaration of Helsinki.

13.1.1 Regulatory Authority Approvals

The sponsor or designee will submit the study protocol plus all relevant study documents to concerned regulatory agencies for approval prior to the study start. No patient will be admitted to the study until appropriate regulatory approval of the study protocol has been received.

Each investigator must complete a Form FDA 1572 (or equivalent) and provide the completed form according to written instructions to the sponsor (or designee). Each investigator must submit to the sponsor (or designee) financial disclosure information according to national law and/or local regulations.

U.S.-generated data will be handled in accordance with the Health Information Portability and Accountability Act (HIPAA). The trial will be registered on regionally relevant registries, including www.clinicaltrials.gov, EudraCT, and the Spanish Clinical Studies Registry using the Protocol Registration System.

13.1.2 Independent Ethics Committee/Institutional Review Board

This protocol and any material to be provided to the patient (such as advertisements, patient information sheets, drug dosing diaries, or descriptions of the study used to obtain informed consent) will be submitted by the investigator to an IEC/IRB. This also applies to protocol amendments.

Clovis Oncology will supply relevant data for the investigator to submit the study protocol and additional study documents to the IEC/IRB. The principal investigator will submit the study protocol for review and approval by an IEC/IRB, according to national law and/or local regulations, and will provide the IEC/IRB with all appropriate materials.

Verification of the IEC's/IRB's unconditional approval of the study protocol and the written informed consent form will be transmitted to Clovis Oncology. This approval must refer to the study by exact study protocol title and number, identify the documents reviewed, and state the date of the review.

No patient will be admitted to the study until appropriate IEC/IRB approval of the study protocol has been received, the investigator has obtained the signed and dated informed consent form, and the sponsor is notified.

The principal investigator will submit appropriate reports on the progress of the study to the IEC/IRB at least annually in accordance with applicable national law and/or local regulations and in agreement with the policy established by the IEC/IRB and sponsor.

The IEC/IRB must be informed by the principal investigator of all subsequent study protocol amendments and of SAEs or SUSARs occurring during the study that are likely to affect the safety of the patients or the conduct of the study.

13.2 Confidentiality of Information

The investigator must assure that patients' anonymity is strictly maintained and that their identities are protected from unauthorized parties. Only patient initials and an identification code (i.e., not names) should be recorded on any form submitted to the sponsor and the IRB. The investigator must record all screened and enrolled patients in the eCRF. The investigator must have a list where the identity of all treated patients can be found.

The investigator agrees that all information received from Clovis Oncology, including, but not limited to, the Investigator's Brochure, this protocol, eCRFs, the protocol-specified treatment, and any other study information, remain the sole and exclusive property of the sponsor during the conduct of the study and thereafter. This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from the sponsor. The investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study center to any third party or otherwise into the public domain.

13.3 Patient Informed Consent

All information about the clinical study, including the patient information and the informed consent form, is prepared and used for the protection of the human rights of the patient according to ICH GCP guidelines and the Declaration of Helsinki.

It is the responsibility of the investigator to obtain signed informed consent forms from each patient participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study and prior to undertaking any study-related procedures.

The informed consent form, prepared by the investigator with the assistance of the sponsor, must be approved along with the study protocol by the IEC/IRB and be acceptable to the sponsor.

The patient must be provided with the patient information and informed consent form consistent with the study protocol version used and approved by the relevant IEC/IRB. The informed consent form must be in a language fully comprehensible to the prospective patient. Patients (and/or relatives, guardians, or legal representatives, if necessary) must be given sufficient time and opportunity to inquire about the details of the study and to discuss and decide on their participation in the study with the investigator concerned. The patient and the person explaining about the study and with whom they discuss the informed consent will sign and date the informed consent form. A copy of the signed informed consent form will be retained by the patient and the original will be filed in the investigator file unless otherwise agreed.

13.4 Study Monitoring

On behalf of Clovis Oncology, a CRO monitor will contact and visit the investigator at the study center prior to the entry of the first patient (unless Clovis or the CRO has worked with the center recently, in which case this initial visit maybe waived) and at predetermined appropriate intervals during the study until after the last patient is completed. The monitor will also perform a study closure visit. Visits may also be conducted by Clovis Oncology personnel.

In accordance with ICH GCP guidelines, the investigator must ensure provision of sufficient time, reasonable space, and adequate qualified personnel for the monitoring visits. The visits are for the purpose of verifying adherence to the study protocol and the completeness, consistency, and accuracy of data entered on the eCRF and other documents.

The investigator will make all source data (i.e., the various study records, the eCRFs, laboratory test reports, other patient records, drug accountability forms, and other pertinent data) available for the monitor and allow access to them throughout the entire study period. Monitoring is done by comparing the relevant site records of the patients with the entries on the eCRF (i.e., source data verification). It is the monitor's responsibility to verify the adherence to the study protocol and the completeness, consistency, and accuracy of the data recorded on the eCRFs.

By agreeing to participate in the study, the investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of the monitoring visits are resolved. Contact information for the study monitor is located in the investigator file. Representatives from Clovis Oncology may also contact and visit the investigators and monitor data during the study.

13.5 Case Report Form

The data will be collected using an electronic data capture (EDC) system by remote data entry on eCRFs. Sites will receive training on the EDC system. All users will be supplied with unique login credentials.

Prior to study start, the investigator will prepare a list showing the signature and handwritten initials of all individuals authorized to make or change entries on eCRFs. This "study center personnel and delegation list" must be kept current throughout the study.

For each patient enrolled, an eCRF must be completed and reviewed by the principal investigator or co-investigator within a reasonable time period (< 2weeks) after data collection. This also applies to records for those patients who fail to complete the study. If a patient withdraws from the study, the reason must be noted on the eCRF. If a patient is withdrawn from the study because of a treatment-limiting AE, thorough efforts should be made to clearly document the outcome.

All laboratory data and investigator observations on the results and any other clinically significant test results must be documented on eCRFs.

Full information regarding electronic data capture and completing eCRFs is included in the investigator files. All questions or comments related to electronic capture should be directed to the assigned monitor.

13.6 Study Termination and Site Closure

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange discontinuation procedures. In terminating the study, Clovis Oncology and the investigator will assure that adequate consideration is given to the protection of the patients' interests.

Clovis Oncology reserves the right to discontinue the study at any time for medical or administrative reasons. When feasible, a 30 day written notification will be given.

The entire study will be stopped if:

- The protocol-specified treatment is considered too toxic to continue the study.
- Evidence has emerged that, in the opinion of the sponsor or the investigator(s), makes the continuation of the study unnecessary or unethical.
- The stated objectives of the study are achieved.
- The sponsor discontinues the development of rucaparib.

Regardless of the reason for termination, all data available for the patient at the time of discontinuation of follow-up must be recorded on the eCRF. All reasons for discontinuation of treatment must be documented. In terminating the study, the investigator will ensure that adequate consideration is given to the protection of the patients' interests.

13.7 Modification of the Study Protocol

Protocol amendments, except when necessary to eliminate an immediate hazard to patients, must be made only with the prior approval of Clovis Oncology. Agreement from the investigator must be obtained for all protocol amendments and amendments to the informed consent document. The IEC/IRB must be informed of all amendments and give approval prior to their implementation. The sponsor will submit any study protocol amendments to the concerned regulatory authorities for approval and keep the investigator(s) updated as detailed in the ICH GCP guidelines.

13.8 Retention of Study Documents

The study site will maintain a study file, which should contain, at minimum, the Investigator's Brochure, the protocol and any amendments, drug accountability records, correspondence with the IEC/IRB and Clovis Oncology, and other study-related documents.

The investigator agrees to keep records and those documents that include (but are not limited to) the identification of all participating patients, medical records, study-specific source documents, source worksheets, all original signed and dated informed consent forms, copies of all eCRFs,

query responses, and detailed records of drug disposition to enable evaluations or audits from regulatory authorities and Clovis Oncology or its designees.

The investigator shall retain records required to be maintained for a period of 5 years following the date a marketing application in an ICH region is approved for the drug for the indication for which it is being investigated or, if no application is to be filed or if the application is not approved for such indication, until at least 5 years after the investigation is discontinued. However, these documents should be retained for a longer period if required by the applicable regulatory requirement(s) or if needed by Clovis Oncology. In addition, the investigator must make provision for the patients' medical records to be kept for the same period of time.

No data should be destroyed without the agreement of Clovis Oncology. Should the investigator wish to assign the study records to another party or move them to another location, Clovis Oncology must be notified in writing of the new responsible person and/or the new location. Clovis Oncology will inform the investigator, in writing, when the trial-related records are no longer needed.

Patients' medical records and other original data will be archived in accordance with the archiving regulations or facilities of the investigational site.

13.9 Clinical Study Report

A clinical study report will be prepared under the responsibility and supervision of Clovis Oncology and signed by the sponsor's chief medical officer, thereby indicating their agreement with the analyses, results, and conclusions of the clinical study report.

13.10 Study Publication

All data generated from this study are the property of Clovis Oncology and shall be held in strict confidence along with all information furnished by Clovis Oncology. Independent analysis and/or publication of these data by the investigator(s) or any member of their staff are not permitted without the prior written consent of Clovis Oncology. Written permission to the investigator will be contingent on the review by Clovis Oncology of the statistical analysis and manuscript, and will provide for nondisclosure of Clovis Oncology confidential or proprietary information. In all cases, the parties agree to submit all manuscripts or abstracts to all other parties 30 days prior to submission. This will enable all parties to protect proprietary information and to provide comments based on information that may not yet be available to other parties.

13.11 Quality Assurance Audits

An audit visit to clinical centers may be conducted by a quality control auditor appointed by Clovis Oncology. The purpose of an audit, which is independent of and separate from routine monitoring or quality control functions, is to evaluate trial conduct and compliance with the protocol, SOPs, ICH GCPs, and the applicable regulatory requirements. The investigator and the sponsor may also be subject to an inspection by FDA, European Regulatory authorities, or other applicable regulatory authorities at any time. The auditor and regulatory authorities will require

authority from the investigator to have direct access to the patients' medical records. It is important that the investigator(s) and their staff cooperate with the auditor or regulatory authorities during this audit or inspection.

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15 APPENDICES

Appendix A. List of Candidate Genes That May Comprise an HRD Signature

Appendix B. Response Evaluation Criteria in Solid Tumors Criteria

Appendix C. Eastern Cooperative Oncology Group (ECOG) Performance Status Scale

Appendix D. Inhibitors and Inducers of CYP1A2 and CYP3A

Appendix E. Gynecological Cancer Intergroup (GCIG) Guidelines for Response by CA-125

15.1 Appendix A

List of Candidate Genes That May Comprise an HRD Signature

Note: this list may be revised prior to initiation of the trial and/or prior to final analysis.

BRCA	nbHRD	
BRCA1	*ATM*	*FANCL*
BRCA2	*ATR*	*FANCM*
	ATRX	*MRE11A*
	BARD1	*NBN*
	BLM	*PALB2*
	BRIP1	*RAD50*
	CHEK1	*RAD51*
	CHEK2	*RAD51B*
	FANCA	*RAD51C*
	FANCC	*RAD51D*
	FANCD2	*RAD52*
	FANCE	*RAD54L*
	FANCF	*RPA1*
	FANCG	
	FANCI	

15.2 Appendix B

Response Evaluation Criteria in Solid Tumors Criteria

The RECIST guidelines (Version 1.1) are described in Eisenhauer (2009) and at http://www.eortc.be/Recist/Default.htm.[50] A short summary is given below.

Measurable Disease:

Tumor lesions: measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) with the following:

- A minimum size of 10 mm by CT scan (CT scan thickness no greater than 5 mm)
- A minimum size of 10 mm caliper measurement by clinical exam (lesions that cannot be accurately measured with calipers should be recorded as nonmeasurable)
- A minimum size of 20 mm by chest X-ray

All tumor measurements must be recorded n millimeters (or decimal fractions of centimeters).

Malignant lymph nodes: to be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be not greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Nonmeasurable Disease:

All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), as well as truly nonmeasurable lesions, are considered nonmeasurable disease. Lesions considered truly nonmeasurable include leptomeningeal disease, ascites, pleural/pericardial effusions, inflammatory breast disease, lymphangitic involvement of skin and lung, and abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Bone Lesions

Bone lesions, cystic lesion, and lesions previously treated with local therapy require particular comment. Bone scan, PET scan, or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.

Lytic bone lesions or mixed lytic–blastic lesions with identifiable soft tissue components that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.

Blastic bone lesions are nonmeasurable.

Cystic Lesions

Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor nonmeasurable) because they are, by definition, simple cysts.

Cystic lesions thought to represent cystic metastases can be considered as measurable lesions if they meet the definition of measurability described above. However, if noncystic lesions are present in the same patient, these are preferred as target lesions.

Lesions with Prior Local Treatment

Tumor lesions situated in a previous irradiated area or in an area subjected to other locoregional therapy are usually not considered measurable unless there has been demonstrated progression in the lesion.

Target Lesions

All measurable lesions up to a maximum of two lesions per organ and five lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

Nontarget Lesions

RECIST criteria require unequivocal quantification of the changes in tumor size for adequate interpretation of the sum of target lesions. Consequently, when the boundaries of the primary are difficult to delineate, this tumor should not be considered a target lesion.

Guidelines for Evaluation of Measurable Disease

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

Evaluation of Target Lesions

Complete Response	Disappearance of all target lesions. Any pathological lymph nodes (whether target or nontarget) must have reduction in short axis to <10 mm.
Partial Response	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD.
Stable Disease	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.
Progressive Disease	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesions is also considered progression.

Evaluation of Nontarget Lesions

Complete Response	Disappearance of all nontarget lesions and normalization of tumor marker level.
Stable Disease/Incomplete Response	Persistence of one or more nontarget lesion(s) or/and maintenance of tumor marker level above the normal limits.
Progressive Disease	Appearance of one or more new lesions and/or unequivocal progression of existing nontarget lesions.

If tumor markers are initially above the institutional ULN, they must normalize for a patient to be considered a complete responder.

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Evaluation of Best Overall Response			
Target Lesions	**Nontarget Lesions**	**New Lesions**	**Overall Response**
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not evaluated	No	PR
SD	Non-PD or not evaluated	No	SD
Not Evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD
NE = Not evaluable.			

Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having symptomatic deterioration. Every effort should be made to document the objective progression, even after discontinuation of treatment.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When evaluation of CR depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspiration/biopsy) prior to confirming the complete response status.

Confirmatory Measurement/Duration of Response

Confirmation

CT scans are required at screening and every 8 weeks (± 4 days) thereafter. Patients who have been on study at least 18 months, may decrease the frequency of disease assessments to every 16 (±2) weeks. If an initial CR or PR is noted , confirmatory scans must be performed at least 4 weeks later.

Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or PD is objectively documented (taking as reference for PD the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of Stable Disease

SD is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

15.3 Appendix C

Eastern Cooperative Oncology Group (ECOG) Performance Status Scale

ECOG Performance Status	
0	Fully active, able to carry on all predisease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (e.g., light house work or office work).
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

In the event performance status is assessed by the Karnofsky Performance Status scale, the following conversion chart applies.

Karnofsky Performance Status			ECOG Performance Status
General Description	**Score**	**Specific Description**	**Score**
Able to carry on normal activity and to work; no special care needed	100	Normal; no complaints; no evidence of disease	0
	90	Able to carry on normal activity; minor signs or symptoms of disease	1
	80	Normal activity with effort; some signs or symptoms of disease	
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed	70	Cares for self, unable to carry on normal activity or to do active work	2
	60	Requires occasional assistance, but is able to care for most of personal needs	
	50	Requires considerable assistance and frequent medical care	3
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly	40	Disabled; requires special care and assistance	
	30	Severely disabled; hospital admission is indicated although death no imminent	4
	20	Very sick; hospital admission necessary; active supportive treatment necessary	
	10	Moribund; fatal processes progressing rapidly	
	0	Dead	5

15.4 Appendix D

Inhibitors and Inducers of CYP1A2 and CYP3A

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#classInhibit

CYP Enzyme	Strong Inhibitor (Avoid)	Moderate Inhibitor (Caution)
CYP1A2	Ciprofloxacin Enoxacin Fluvoxamine	Methoxsalen Mexiletine Phenylpropanolamine Thiabendazole Zileuton
CYP3A	Boceprevir Clarithromycin Conivaptan Grapefruit juice Indinavir Itraconazole Ketoconazole Lopinavir/Ritonavir Mibefradil Nefazodone Nelfinavir Posaconazole Ritonavir Saquinavir Telaprevir Telithromycin Voriconazole	Amprenavir Aprepitant Atazanavir Ciprofloxacin Darunavir/Ritonavir Diltiazem Erythromycin Fluconazole Fosamprenavir Grapefruit juice* Imatinib Verapamil

* The effect of grapefruit juice varies widely among brands and is concentration-, dose-, and preparation-dependent. Patients should be instructed to avoid grapefruit juice in this study.

CYP Enzyme	Strong Inducer (Avoid)	Moderate Inducer (Caution)
CYP1A2	N/A	Montelukast Phenytoin Smoking
CYP3A	Avasimibe Carbamazepine Phenytoin Rifampin St. John's Wort	Bosentan Efavirenz Etravirine Modafinil Nafcillin

15.5 Appendix E

Modified Gynecological Cancer Intergroup (GCIG) Guidelines for Response Using CA-125

Adapted from Rustin et al., Int J Gynecol Cancer. 2011[51]

GCIG CA 125 definitions are available at http://gcig.igcs.org/CA-125.html.

To be evaluable for response by CA-125 requires an elevated baseline value of at least twice the upper limit of normal and at least two additional samples after the start of treatment.

A response to CA-125 has occurred if there is at least a 50% decrease from baseline:

1. in a sample collected after initiation of study treatment AND

2. that is confirmed in a subsequent sample collected ≥21 days after the prior sample. The absolute value of this confirmatory sample must be ≤110% of the prior sample.

The date when the first sample with a 50% decrease from baseline is observed is the date of the CA-125 response.

In patients who have measureable disease by RECIST v1.1 and CA-125, the date of response will be the date of the earlier of the two events. When assessing progression, the objective change in tumor size should be used for treatment decisions. For example, if a patient has a reduction in measurable disease, but an increase in CA-125 that suggests progression, treatment should continue.

STATISTICAL ANALYSIS PLAN

A Phase 2, Open-Label Study of Rucaparib in Patients with Platinum Sensitive Relapsed High-Grade Serous Ovarian Cancer to Characterize the Relationship between Efficacy and Homologous Recombination Repair Deficiency

PROTOCOL NUMBER: CO-338-017

DATE FINAL: DRAFT

SPONSOR: Clovis Oncology
2525 28th St.
Boulder, CO 80301
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TABLE OF CONENTS

LIST OF IN-TEXT TABLES

ABBREVIATIONS AND SPECIALIST TERMS

AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
ANA	Antinuclear antibody
ANCA	Serum antineutrophilic cytoplasmic antibody
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
ATC	Anatomical Therapeutical Chemical (coding)
BP	Blood pressure
BRCA	breast cancer genes
BSA	Body surface area
CA-125	Cancer antigen 125
CI	Confidence interval
cm	centimeter
CR	Complete response
CRF	Case report form
CRP	C-reactive protein
C_{max}	Peak (maximum) plasma concentration
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DOR	Duration of response
DRS-P	Disease related symptoms-physical subscale
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
FACT	Functional Assessment of Cancer Therapy
FCBP	Female of child bearing potential
FOSI-18	FACT-Ovarian Symptom Index-18
GCIG	Gynecologic Cancer Intergroup
GI	Gastrointestinal
HGSOC	High Grade Serous Ovarian Cancer
HRD	homologous recombination deficiency
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
invPFS	Progression-free survival, investigator assessed

ITT	Intent to treat
kg	Kilogram
LDH	Lactate dehydrogenase
LDT	Laboratory developed test
MAR	Missing at random
MedDRA	Medical Dictionary for Drug Regulatory Activities
nbHRD	Non-*BRCA* HRD
NCCN	National Comprehensive Cancer Network
NGS	Next generation sequencing
NMAR	Not missing at random
ORR	Objective response rate
OS	Overall survival
PD	Pharmacodynamic
PFS	Progression-free survival
PK	Pharmacokinetics
PO	Oral (*per os*)
PR	Partial response
PRO	Patient reported outcome
PTE	Proportion of treatment effect
QD	Once daily
RBC	Red blood cell
RECIST	Response Evaluation Criteria In Solid Tumors
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
TEAEs	Treatment-emergent adverse events
TTO	Time Trade-Off
UK	United Kingdom
ULN	Upper limit of normal
VAS	Visual Analog Scale
WBC	White blood cell
WHO	World Health Organization
Wt	Wild type

1 INTRODUCTION

This document describes the statistical analyses and data presentations to be performed for Clovis Oncology's protocol CO-338-017. This statistical analysis plan (SAP) provides a comprehensive and detailed description of the strategy, rationale, and statistical techniques to be used to assess the efficacy and safety of rucaparib (CO-338) in patients with relapsed high-grade serious ovarian cancer (HGSOC) following platinum sensitive relapse (PSR) and subsequent response to platinum based therapy.

The purpose of the SAP is to ensure the credibility of the study findings by specifying the statistical approaches to the analysis of study data prior to database lock for the final analysis. This SAP provides additional details concerning the statistical analyses that were outlined in the original protocol dated 7 May 2013 and protocol Amendment 1 dated 19 Aug 2013, Amendment 2 dated 9 May 2014, and Amendment 2.1 dated 30 May 2014, and Amendment 3 [redacted].

All statistical analyses detailed in this SAP will be conducted using SAS® Version 9.3 or higher.

2 OVERALL STUDY DESIGN, OBJECTIVES, AND ENDPOINTS

2.1 Study Objectives and Endpoints

Table 1. Primary, Secondary, and Exploratory Objectives and Endpoints

Primary Objectives	**Primary Endpoints**
To determine the PFS of rucaparib in molecularly-defined HRD subgroups	Disease progression (RECIST v1.1) as assessed by investigator, or death from any cause (invPFS) in molecularly-defined subgroups
Secondary Objectives	**Secondary Endpoints**
To evaluate response	ORR by RECIST v1.1 and GCIG CA-125 criteria
To assess duration of response (DOR)	DOR by RECIST v1.1
To evaluate safety and tolerability of rucaparib	The incidence of adverse events (AEs), clinical laboratory abnormalities, and dose modifications
To evaluate steady state trough PK	Trough (C_{min}) level rucaparib concentrations
Exploratory Objectives	**Exploratory Endpoints**
To assess efficacy in molecularly-defined HRD subgroups as defined by HRR gene alterations	PFS and/or ORR by RECIST v1.1 and GCIG CA-125 criteria. HRD subgroups as defined by HRR gene alterations
To explore HRD in tumor tissue over time	Changes HRD (LOH and gene alterations) in fresh biopsy versus archival tumor tissue samples
To explore whether the BROCA panel can identify mutations in additional HRR genes that may be associated with efficacy	PFS and/or ORR by RECIST v1.1 and GCIG CA-125 criteria in relation to HRR gene mutations identified in BROCA
[redacted]	[redacted] and PFS and/or ORR by RECIST v1.1 and GCIG CA-125 criteria
To explore if a gene expression signature for HRD correlates with response	PFS and/or ORR by RECIST v1.1 and GCIG CA-125 criteria in relation to gene signature defined by a gene expression profiling assay
[redacted]	[redacted] in relation to PFS and/or ORR by RECIST v1.1 and GCIG CA-125 criteria

2.2 Trial Design

This is an open-label study of rucaparib in patients with platinum-sensitive, relapsed, high-grade epithelial ovarian (serous or endometrioid histology), fallopian tube, or primary peritoneal cancer. The purpose of this study is to define a molecular signature of HRD in ovarian cancer that correlates with response to rucaparib and enables selection of appropriate ovarian cancer patients for treatment with rucaparib. The HRD signature will be based on an association between genomic scarring, as defined by a *BRCA* mutation or high level of loss of genomic heterozygosity (LOH), observed in a patient's tumor and observed clinical benefit from rucaparib treatment. In addition, the sequence of other HRR genes will be examined to determine if alterations in these genes are associated with response to rucaparib (**Table 3**).

In addition, the safety and efficacy is going to be further evaluated in the subgroup of patients with BRCA mutation. The study schema can be found in the latest amendment. amenements.

2.3 Treatments and Assignment to Treatments

All eligible patients will receive rucaparib.

2.4 Sample Size Justification

Initially approximately 180 patients will be enrolled to ensure each HRD subgroup, tBRCA (HRD related to a deleterious *BRCA1* or *BRCA2* gene mutation in tumor tissue) nbHRD (LOH^+ with no *BRCA1/2* mutation), and biomarker negative (no *BRCA1*, *BRCA2* mutations, and LOH^-) will contain an adequate number of patients. Other than the initial cap on known *gBRCA* and subsequent cap on BRCA patients, (n=15 and n=50, respectively), there will be no specific requirement to enroll defined numbers of patients into each planned subgroup. The likely size of each subgroup has been estimated based on: a) frequencies of HRD-associated abnormalities at initial diagnosis as reported in the literature and b) the hypothesis that the inclusion criterion of sensitivity to platinum following the most recent line of platinum therapy will enrich the population for patients with tumors harboring alterations of HRD pathway genes (i.e., that the frequency will be greater than that described in the newly-diagnosed population).

Once an adequate number of patients have been enrolled to represent each of the HRD subgroups (approx. 180 patients) then 50 additional patients with a known BRCA mutation (germline or somatic) will be enrolled

Table 2 provides estimated subgroups sizes.

Table 2 Estimated HRD Subgroup Sizes

HRD Subgroup	Expected Frequency at Diagnosis [1]	Estimated Frequency with Enrichment for Platinum Sensitivity	Estimated Number of Patients

tBRCA	21%	30%	15 with known deleterious *gBRCA* mutation (fixed) ***plus*** 20 – 25 with somatic *BRCA* mutation ***plus*** 5 – 25 additional with newly diagnosed *gBRCA* mutation ***plus*** 50 with *BRCA* mutation
nbHRD	22 – 32%	30 – 50%	50 – 90
Biomarker Negative	60 – 70%	20 – 40%	36 – 72

Enrollment of patients known *a priori* to harbor a *gBRCA* mutation classified as deleterious (pathogenic), suspected deleterious, or favor deleterious (or the equivalent interpretation of any of these) on the most recent assessment by a testing laboratory will be initially limited to 15. Fifteen patients with a known *gBRCA* mutation are sufficient to establish that the frequency of *gBRCA* mutation reversions is low. If none of the patients with a known *gBRCA* mutation is shown to have a reversion between archival tissue and tumor tissue collected at screening, then the frequency of *gBRCA* reversions is likely less than 20% as the upper bound of the 90% confidence interval (CI) is 18%. Additional patients may be identified as having a deleterious *BRCA* mutation in tumor tissue, therefore the initial tBRCA subgroup will likely contain at least 40 patients and then once the nbHRD and Biomarker negative groups have been fully enrolled, an additional 50 patients with BRCA will be enrolled, leading to a total of at least 90 tBRCA patients.

The benefit of rucaparib is expected to be the greatest in the tBRCA group, followed by the nbHRD group, and lowest in patients that are biomarker negative. This study will provide evidence as to whether the benefit of rucaparib is clinically meaningful in each of these subgroups, and particularly in the nbHRD subgroup.

With approximately 230 patients enrolled in the study, the comparison of any 2 subgroups will likely contain about 100 patients. Therefore with 100 patients, there is 80% power at a 2-sided 10% significance level to detect a difference in PFS distributions assuming the hazard ratio between 2 subgroups is 0.50,

The reason for a sample size of at least 90 patients in the subgroup of patients with a known BRCA mutation (germline or somatic) is to better characterize the efficacy and safety in this subgroup. Response rates in the range of 30% to 40% have been observed in ovarian cancer patients with a *gBRCA* mutation treated with a PARP inhibitor [**Error! Reference source not found.**, **Error! Reference source not found.**]. The table below provides 95% CIs for observed response rates of 30%, 40%, 50%, and 60% assuming a sample size of 90 patients within the subgroup of patients with known BRCA.

Confidence Intervals for Observed Response Rates (ORR)

ORR(%)	[95% CI]
30	20.8,40.6
40	29.8,50.9
50	39.2, 60.7
60	49.1, 70.2

CI=Confidence intervals of ORR using Clopper-Pearson methodology.

Therefore, an ORR of 30% would show similar response rate as other PARP inhibitors and an observed response rate of 50% would show a significant improved response rate compared to other PARP inhibitors.

3 GENERAL ANALYSIS CONVENTIONS

The summary tables will be presented for all treated patients and by the subgroups defined by HRD status (BRCA, nbHRD, and biomarker negative).

Quantitative variables will typically be summarized using frequencies and percentages for appropriate categorizations and may also be summarized using descriptive statistics. For variables summarized with descriptive statistics, the following will be presented: N, mean, standard deviation, median, minimum and maximum. Categorical variables will be presented using frequencies and percentages. The Kaplan-Meier methodology will be used to summarize time-to-event variables. If estimable, the 25th, 50th (median), and 75th percentiles will be presented along with the Kaplan-Meier estimates of event rates at 6-month intervals. The number of patients with events and the number of censored patients will also be presented.

All data will be used to their maximum possible extent but without any imputations for missing data.

Unless otherwise specified, baseline is defined as the last measurement on or prior to the first day of study drug administration.

4 ANALYSIS POPULATIONS

Safety Population: The safety population will consist of all patients who received at least one dose of protocol-specified treatment.

Efficacy Population: The safety population will consist of all patients who received at least one dose of protocol-specified treatment.

5 PATIENT DISPOSITION

Patient disposition (analysis population allocation, entered, discontinued, along with primary reason for discontinuation) will be summarized using frequency counts, and the corresponding percentages.

6 INCLUSION / EXCLUSION VIOLATIONS

The number of patients that violate each inclusion or exclusion criteria will be summarized with frequencies and percentages or provided in a patient listing.

7 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

All demographic and baseline characteristics will be summarized for the safety population.

7.1 Definition of HRD Subgroups

The following sections define various methods for classifying patients as positive for homologous recombination deficiencies. The primary methodology of interest is based on *BRCA* mutations and the percentage of the genome with loss of heterozygosity (LOH). The other definitions presented will be considered exploratory analyses of HRD. Additional algorithms and/or technologies not presented in this SAP may also be explored in this study. The assay results from the most recent tumor biopsy will be used for each patient.

7.1.1 Tumor BRCA Mutation and Percentage of Genome with LOH

The primary HRD subgroup of interest is defined by the following HRD definition:

> A patient will be categorized as positive for HRD if the patient has a pathogenic *BRCA* mutation and/or the percent of the patient's tumor genome with loss of heterozygosity (LOH) is [redacted]. For further rationale around this definition see Appendix A.

Sensitivity analyses of this definition will be performed to assess whether [redacted] is the optimal cut-off for discriminating the efficacy of rucaparib.

7.1.2 Number of Long LOH Regions

Tissue BRCA mutations along with the number of long regions of LOH in the tumor genome will also be analyzed as an exploratory definition of HRD. [redacted].

Predefined Lists of Genes

A deleterious alteration within a pre-specified list of genes can also be used to categorize patients as HRD positive. The columns in the following table specify 4 groups of genes that will be used to define HRD subgroups in this study (HRD-G1, HRD-G2, HRD-G3, and HRD-G4).

Table 3 Predefined Lists of Genes

Gene	HRD-G 1 (TCGA)	HRD-G 2 (Patent TCGA)	HRD-G 3 (siRNA)	HRD-G 4 (Patent siRNA)
BRCA1	X	X	X	
BRCA2	X	X	X	
FANCA	X	X	X	
PTEN	X			
RAD51	X	X	X	
ATM	X	X	X	

CHEK2/CHK2	X	X		
RAD50	X	X		
MRE11/MRE11A	X	X		
RPA1	X	X	X	
CHEK1/CHK1	X	X	X	
FANCC	X	X		
RAD51D	X	X		
ATR	X	X	X	
FANCF	X	X	X	
FANCD2	X	X	X	
FANCE	X	X	X	
FANCG	X	X	X	
RAD52	X	X	X	
RAD51C	X	X	X	
FANCM	X		X	
ATRX	X		X	
BLM	X		X	
PALB2	X		X	
RAD54L	X			
BRIP1	X			
FANCI	X		X	
NBN	X		X	
FANCL	X			
BARD1	X		X	
RAD51B	X		X	

7.2 Demographics

The demographic variables will be summarized with frequency tabulations that will focus on identifying the extreme values of the distributions. Descriptive statistics may also be used to summarize the quantitative variables. The demographic variables presented will include age, height, weight, gender, race, and ECOG Performance Status using the following categorizations:

- Age (years): ≤ 50, 51-60, 61-70, 71-80, 81-90, > 90;
- Height (cm): ≤ 75, > 75-100, > 100-125, > 125-150, > 150-175, > 175;
- Weight (kg): ≤ 50, > 50-75, > 75-100, > 100-125, > 125-150, > 150;
- Race: American Indian or Alaska Native, Asian, Black, Native Hawaiian or Other Pacific Islander, White, Other
- ECOG Performance Status: 0, 1, ≥2
- Region: North America, Western Europe, and Israel

These categorizations may be adjusted if the majority of the data lies in only 2 or 3 of the categories.

7.3 Baseline Clinical Characteristics

The following variables will be summarized with frequency tabulations

- Time since diagnosis of HGSOC (months): ≤ 3, > 3-6, > 6-12, > 12-24, > 24;
- Baseline laboratory parameters: graded based on CTCAE;
- HRD status;
- Number of prior platinum therapies.

Descriptive statistics may also be used to summarize these variables.

7.4 Medical History

Medical history data will be summarized using frequency tabulations by system organ class and preferred term.

8 STUDY DRUG EXPOSURE AND COMPLIANCE

The following variables will be summarized:

- Number of cycles initiated
- Duration of treatment
- Number of patients with at least one dose reduction, delay or increase;

The duration of treatment will be calculated as the number of days from the first dose of study drug to the day of the last dose of study drug + 1. The number of patients with at least one dose delay or reduction will be summarized with frequencies and percentages.

9 PRIOR AND CONCOMITANT MEDICATIONS

All concomitant treatments documented during the study period will be summarized in frequency tabulations. Prior/concomitant medication coding will utilize World Health Organization (WHO) Drug version March 1, 2007.

Separate data summaries of prior medications will be provided. Prior medications will be defined as those medications with both a start and a stop date that is before the day of the first dose of study drug administration. If either the start date and/or the stop date of the medication is missing so that it is unclear whether the medication was stopped prior to first dose of study drug administration then the medication will be included in the summary of the concomitant medications.

10 EFFICACY VARIABLES

10.1 Primary Efficacy Variable

The primary efficacy endpoint is disease progression according to RECIST v1.1, as assessed by the investigator or death from any cause, in molecularly-defined HRD subgroups.
Secondary Efficacy Variables

Secondary variables include:

- ORR assessed by RECIST v1.1
- ORR assessed by GCIG CA-125 response criteria
- DOR by RECIST v1.1
- Safety and tolerability
- PK at steady state trough levels

10.2 Exploratory Efficacy Variables

Exploratory variables include:

- PFS and/or ORR by RECIST v1.1 and GCIG CA-125 criteria. HRD subgroups as defined by HRR gene alterations.
- Changes in HRD (LOH and gene alterations) in fresh biopsy versus archival tumor tissue samples

11 EFFICACY ANALYSIS

All efficacy evaluations will be conducted using the safety population.

11.1 Primary Efficacy Analysis

The primary efficacy endpoint is PFS will be calculated as 1+ the number of days from the first dose of study drug to disease progression, as determined by the investigator or death due to any cause, whichever occurs first. Patients without a documented event of progression will be censored on the date of their last adequate tumor assessment (i.e., radiologic assessment) or date of first dose of study drug if no tumor assessments have been performed.

11.2 Secondary Efficacy Analyses

Secondary efficacy analyses will be based on the safety population or subgroups thereof.

11.2.1 Overall Response Rate Assessed by RECIST and GCIG CA-125 Criteria

The endpoint of ORR defined as a best response of CR or PR using RECIST (**Error! Reference source not found.**) will be summarized with frequencies and percentages in the safety population. As a supportive analysis, the ORR will also be evaluated in the patients with both a baseline tumor assessment and at least one post-baseline tumor assessment.

The endpoint of CA-125 overall response rate defined as a 50% reduction in CA-125 as assessed by GCIG criteria will be summarized with frequencies and percentages in the safety population. As a supportive analysis, the CA-125 response rate will also be evaluated in the patients evaluable for a CA-125 response as defined in **Error! Reference source not found.** of the protocol.

ORR will be reported separately and together for RECIST and GCIG. The combined ORR will be assessed as indicated in Table 4.

Table 4 Overall Response by RECISTError! Reference source not found. and GCIG CA-125 CriteriaError! Reference source not found.

RECIST Response	**GCIG CA-125 Response**	**RECIST + GCIG CA-125 Combined**
CR (requires normalization of CA-125)	CA-125 within normal range	Response
PR	Response	Response
PR	No Response	Response
SD	Response	Response
SD	No Response	No Response
PD	Response	No Response
PD	No Response	No Response

11.2.2 Overall Response Rate using RECIST

Patients must have both baseline tumor assessment and at least on post baseline tumor assessment in order to be considered evaluable for a RECIST response.

Measurable Lesions

Tumor lesions will be categorized as measurable if they can be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm)
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable)
- 20 mm by chest X-ray
- Lymph nodes will be classified as measurable if the short axis≥15 mm.

Non-measurable Lesions

All other lesions not characterized as measurable, including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include leptomeningeal disease ascites, pleural or pericardial effusion inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal oranomegaly identified by physical exam that is not measureable by reproducible imaging techniques.

The sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response

Response Criteria

A maximum of 5 measurable lesions will be defined as target lesions (at most 2 per organ representing all involved organs). Target lesions will be evaluated based on the following criteria:

Complete Response (CR):	Disappearance of all target lesions. Any pathological lymph nodes (whether target or nontarget) must have reduction in short axis to <10 mm.

Partial Response (PR):	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD.
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.
Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesions is also considered progression.

Non-target lesions are all other lesions not identified as target lesions. Non-Target lesions will be evaluated based on the following criteria:

Complete Response (CR):	Disappearance of all nontarget lesions
Stable Disease (SD)/ Incomplete Response:	Persistence of one or more nontarget lesion(s)
Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing nontarget lesions.

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria as outlined in Table 5.

Table 5. Measurement and Confirmation Criteria

Target Lesions	Non-target lesions	New lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not evaluated	No	PR

SD	Non-PD or not evaluated	No	SD
Not Evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD
NE = Not evaluated "Any" refers to any response, i.e., CR, PR, SD, or PD			

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration."

Confirmatory Measurement

Confirmation of a PR or CR is required by a subsequent tumor assessment no less than 4 weeks later. In the case of SD follow-up measurements must have met the SD criteria at least once after study entry, at a minimum interval of no less than 8 weeks.

11.2.3 Response Rate using GCIG Criteria for CA-125[1]

To be evaluable for response by CA-125 requires two pretreatment samples at least twice the upper limit of normal (>70 iU/mL) and at least two additional samples after the start of treatment.

A response to CA-125 has occurred if after two elevated levels before therapy there is at least a 50% decrease that is confirmed by a fourth sample. The four samples must satisfy the following criteria:

1. The two pretreatment samples must both be at least twice the upper limit of normal and at least 1 day but not more than 3 months apart;

2. At least one of the two pretreatment samples should be within 2 week of starting treatment;

3. The third sample must be ≤ 50% of the second sample;

4. The confirmatory fourth sample must be ≥ 21 days after sample 3 and ≤ 110% of sample 3;

5. Any intervening samples between samples 2 and 3 and between samples 3 and 4 must be ≤ 110% of the previous sample unless considered to be increasing because of tumor lysis.

Patients are not evaluable by CA-125 if they have received mouse antibodies or if there has been medical or surgical interference with their peritoneum or pleura during the previous 28 days.

11.2.4 Duration of Response

The duration of response is measured from the time measurement criteria are met for CR/PR per RECIST or a 50% response in CA-125 (whichever is first recorded) until the first date that recurrent or PD is objectively documented.

The duration of response will also be evaluated separately for CR/PR RECIST responses and for CA-125 responses. In addition, the duration of overall CR will be measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

The duration of response will be summarized with descriptive statistics. Only patients with a response will be included in the summary.

11.3 Exploratory Efficacy Analyses

11.3.1 Additional HRD Subgroups

The primary and secondary efficacy endpoints will be evaluated in the other HRD subgroups outlined in section 7.1 as well as HR gene mutations identified in BROCA.

11.3.2 Association among Matched Archival and Fresh Tumor Tissue Samples

The association in HRD (LOH and gene alterations) between matched archival and fresh tumor samples will be evaluated with frequencies and percentages.

11.3.3 [redacted]

The PFS and/or ORR by RECIST v1.1 and GCIG CA-125 criteria will be evaluated among subgroups of patients defined by varying levels of [redacted].

11.3.4 Evaluation of Germ Line BRCA Mutations

Patients with a HRD alteration (including BRCA) will be classified as having a germ line or somatic alteration based on the methodology developed by Foundation Medicine (FMI). Based on this classification of germ line or somatic alterations, ORR by RECIST v1.1 and GCIG CA-125 criteria will be evaluated between these subgroups within patients with BRCA mutations and HRD alterations.

11.3.5 [redacted]

The mean and/or percentage change from baseline in [redacted] will be summarized with descriptive statistics at each visit and plotted over time for the HRD subgroups.
In order to explore whether other measures of the [redacted] are predictive of outcome with rucaparib therapy, the patients will be divided into a training dataset and a test dataset. The training dataset will consist of approximately the first 50 patients treated in the study. The hypotheses generated using the training dataset will then be prospectively evaluated in the test dataset. The analyses to be evaluated in the test dataset will be documented in a separate analysis plan or incorporated in this analysis plan as an amendment.

12 STATISTICAL / ANALYTICAL ISSUES

12.1 Handling of Dropouts or Missing Data

Patients with an unknown HRD status will only be included in the analyses of the overall safety group.

12.2 Pooling of Centers in Multi-Center Studies

The centers within a given region (e.g., United States versus Rest of World) will be pooled for stratification by region and subgroup analyses by region.

12.3 Multiple Comparison / Multiplicity

No adjustments for multiple comparisons will be made.

12.4 Examination of Subgroups

Subgroup analyses of the primary endpoint based upon age and ECOG will be provided for the primary HRD subgroup and other key exploratory HRD subgroups.

12.5 Interim Analysis

No formal interim analysis of efficacy will be performed.

12.6 Independent Data Monitoring Committee (IDMC)

No formal IDMC will be used since this is an open-label study.

13 SAFETY ANALYSIS

The safety analyses will be performed using the safety population.

13.1 Adverse Events

Adverse events will be classified using the Medical Dictionary for Drug Regulatory Activities (MedDRA) classification system. The severity of the toxicities will be graded according to the NCI CTCAE whenever possible. Treatment-emergent adverse events (TEAEs) are defined as AEs with onset date on or after the date of first dose of study medication until the date of the last study medication dose plus 28 days. Adverse events will be considered treatment-emergent if all or part of the date of onset of the adverse event is missing and it cannot be determined if the adverse event meets the definition for treatment-emergent.

The number and percentage of patients who experienced TEAEs for each system organ class and preferred term will be presented. Multiple instances of the TEAE in each system organ class and multiple occurrences of the same preferred term are counted only once per patient. The number and percentage of patients with at least one TEAE will also be summarized.

Separate tables will be presented as follows:

- All TEAEs;
- TEAEs by CTCAE grade;
- Grade 3 or greater TEAEs;
- Treatment-related TEAEs;
- Serious TEAEs;
- TEAEs with an outcome of death;
- TEAEs leading to discontinuation of study medication;
- TEAEs resulting in interruption of study medication; and
- TEAEs resulting in reduction, delay or interruption of study medication.

The incidence of TEAEs will be summarized by relationship to study drug according to the following categories: "treatment-related," or "not treatment-related". If a patient experiences multiple occurrences of the same AE with different relationship categories, the patient will be counted once, as a relationship category of treatment related.

If a patient experiences multiple occurrences of the same AE with different toxicity grades, the patient will be counted once for the maximum (most severe) toxicity grade. AEs with a missing toxicity grade will be presented in the summary table with a toxicity grade of "Missing." For each toxicity grade, the number and percentage of patients with at least one TEAE of the given grade will be summarized.

Non-TEAEs (pre-treatment and post-treatment) will be presented in the by patient data listings for the safety population.

13.2 Clinical Laboratory Evaluations

Clinical laboratory evaluations include the continuous variables for hematology, serum chemistry, and urinalysis. The laboratory values will be presented in SI units. The on-treatment period will be defined as the time from randomization to 28 days after the last dose of study drug. Laboratory values collected during the on-treatment period will be included in the summary tables. The laboratory values collected after the on-treatment period will only be presented in the data listings.

The summary of laboratory data will include descriptive statistics (N, mean, SD, minimum, median, and maximum) of the maximum, minimum and last value during the on-treatment period. Summaries using descriptive statistics of the change from baseline to the maximum, minimum, and last value during the on-treatment period will also be given.

Supporting laboratory data including normal ranges and abnormal laboratory flags will be provided using by-patient listings. Separate listings will be produced for clinically significant laboratory abnormalities (i.e., those that meet Grade 3 or 4 criteria according to CTCAE Version 4.0).

13.3 Vital Signs

The on-treatment period will be defined as the time from randomization to 28 days after the last dose of study drug. Vital sign measurements collected during the on-treatment period will be included in the summary tables. The vital sign measurements collected after the on-treatment period will only be presented in the data listings.

The summary of vital sign data will include descriptive statistics (N, mean, SD, minimum, median, third quartile and maximum) of the maximum, minimum and last value during the on-treatment period. Summaries using descriptive statistics (N, mean, SD, minimum, median and maximum) of the change from baseline to the maximum, minimum, and last value during the on-treatment period will also be given. The data will be presented separately for each randomized treatment group and overall.

14 REFERENCES

1. Rustin GJS, Vergote I, Eisenhauer E, et al. Definitions for Response and Progression in Ovarian Cancer Clinical Trials Incorporating RECIST 1.1 and CA 125 Agreed by the Gynecological Cancer Intergroup (GCIG). Int J Gynecol Cancer 2011;21:419-23

15 [redacted]